Korean Journal of Environmental Biology

Note

https://doi.org/10.11626/KJEB.2024.42.3.288

Korean J. Environ. Biol.
42(3) : 288-300 (2024)
ISSN 1226-9999 (print)
ISSN 2287-7851 (online)

A report on 22 unrecorded *Actinomycetota* species isolated from freshwater environments in the Republic of Korea

Soo-Yeong Lee, Jaeduk Goh and Ahyoung Choi*

Biological Resources Research Department, Nakdonggang National Institute of Biological Resources (NNIBR), Sangju 37242, Republic of Korea

Contribution to Environmental Biology

- This study identifies 22 previously unrecorded *Actinomycetota* species in freshwater ecosystems of Korea, filling a crucial knowledge gap and aiding biodiversity preservation.
- These new *Actinomycetota* species offer the potential for developing antibiotics, bioactive compounds, and bioremediation agents, supporting sustainable practices and water quality improvement.

***Corresponding author**
Ahyoung Choi
Tel. 054-530-0841
E-mail. aychoi@nnibr.re.kr

Received: 12 July 2024
Revised: 24 August 2024
Revision accepted: 10 September 2024

Abstract: Freshwater environments are rich ecosystems that support diverse microbial communities, including members of the phylum *Actinomycetota* critical for nutrient cycling, organic matter decomposition, and water quality maintenance. *Actinomycetota* known to produce numerous bioactive secondary metabolites are valuable in biotechnology, medicine, and agriculture. Despite their significance, the diversity and distribution of *Actinomycetota* in freshwater habitats, especially in the Republic of Korea, are underexplored. This study aimed to report the isolation and characterization of 22 previously unrecorded bacterial species of *Actinomycetota* from various freshwater environments in Korea. Using standard dilution plating techniques on six different culture media, 22 bacterial strains were isolated, incubated, and characterized based on colony and cellular morphologies, Gram staining, and biochemical properties. Genomic DNA was extracted and the 16S rRNA gene was sequenced to determine species identity using the EzBioCloud service with a cutoff of 98.7% sequence similarity for classification as unreported species. These strains were phylogenetically diverse, belonging to two classes, ten orders, and eighteen genera. This study enhances our understanding of bacterial diversity in freshwater ecosystems and underscores the importance of exploring microbial diversity in underexplored habitats, potentially leading to discovery of novel bioactive compounds. Findings of this study contribute valuable insights into ecological roles and biotechnological potential of *Actinomycetota* in freshwater environments.

Keywords: freshwater environments, unrecorded species, *Actinomycetota*, bacterial diversity

1. INTRODUCTION

Freshwater environments are diverse ecosystems that harbor a wide range of microbial communities, including bacteria that play critical roles in nutrient cycling, organic matter decomposition, and maintaining water quality (Böckelmann *et al.* 2000; Ferreira *et al.* 2020). Among these bacterial communities, members of the phylum *Actinomycetota* are of particular interest due

to their ecological significance and potential applications in biotechnology, medicine, and agriculture (Blin *et al.* 2021; Kim *et al.* 2021; Ngamcharungchit *et al.* 2023; Bruna *et al.* 2024). *Actinomycetes* are known for their ability to produce a vast array of secondary metabolites, including antibiotics, antifungals, and immunosuppressive agents, making them valuable resources for novel bioactive compounds (Goodfellow and Williams 1983; Comba *et al.* 2013; Donald *et al.* 2022). Furthermore, these species play crucial roles in biodegradation and pollutant removal, contributing to environmental remediation processes, that support sustainable environmental management (Bérdy 2005; Slemc *et al.* 2021; Behera and Das 2023).

Despite their importance, the diversity and distribution of *Actinomycetota* in freshwater environments remain underexplored, particularly in regions such as Korea. Previous studies have primarily focused on marine environments and soil, leaving a significant knowledge gap regarding freshwater habitats (Bae *et al.* 2016; Lee *et al.* 2016; Heo *et al.* 2023). The unique physicochemical conditions of freshwater systems, such as rivers, lakes, and reservoirs, offer distinct niches that may support the growth of unrecorded or novel bacterial species.

Actinomycetota is a bacterial phylum that underwent renaming from '*Actinobacteria*' in 2021 (Oren and Garrity 2021). Representing a diverse phylum of Gram-positive bacteria, *Actinomycetota* are characterized by their high G+C content (Gao and Gupta 2012). They inhabit both terrestrial and aquatic environments and play a crucial role in various ecosystems (Servin *et al.* 2008). As of the time of this writing, taxonomic classification based on 16S rRNA gene sequences indicates that the phylum *Actinomycetota* comprises six classes with correct names: *Acidimicrobiia*, *Actinomycetia*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria*, and *Thermoleophilia*. *Actinomycetota* stands out as one of the dominant bacterial phyla, boasting one of the largest bacterial genera, *Streptomyces*. *Streptomyces* and other *Actinomycetota* play crucial roles in soil health by contributing significantly to biological buffering (Donald *et al.* 2022).

In this study, we report the isolation and characterization of 22 previously unrecorded bacterial species belonging to the phylum *Actinomycetota* from various freshwater environments in Korea. This research aims to contribute to the understanding of bacterial diversity in freshwater ecosystems and provide insights into the potential biotechnological applications of these newly identified species. The findings of this study highlight the importance of continued exploration of microbial diversity in underexplored habitats, which could lead to the discovery of novel bioactive compounds and enhance our understanding of microbial ecology.

2. MATERIALS AND METHODS

A total of 22 bacterial strains were obtained from various freshwater environments by employing the standard dilution plating technique on different culture media. The media used included marine agar 2216 (MA), Reasoner's 2A (R2A), 1/10-diluted R2A (1/10 R2A), nutrient agar (NA), 1/10-diluted tryptic soy agar (1/10 TSA), and inorganic salts starch (ISP 4) agar. These media were selected based on their efficacy in supporting the growth of diverse bacterial communities, particularly *Actinomycetota*, which are known for their unique nutrient requirements. The isolates were incubated at 15−25°C for 14 days to accommodate a range of growth temperatures suitable for different bacterial species. After serial dilution spreading, each strain was isolated as a single colony, and the pure cultures were stored as 20% glycerol suspensions at −80°C. Table 1 provides details of the strain IDs, culture media, and incubation conditions.

For 16S rRNA sequence analysis, genomic DNA was extracted from the isolates using the DNeasy Blood & Tissue kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using universal bacterial primers 27F and 1492R and sequenced through Sanger sequencing (Weisburg *et al.* 1991). The nearly full-length 16S rRNA gene sequences obtained were identified using the "16S-based ID" service in EzBioCloud (Yoon *et al.* 2017) with a sequence similarity cutoff set at 98.7%. Strains showing $\geq$98.7% sequence similarity with known bacterial species not previously reported in Korea were classified as unreported species.

Phylogenetic analyses involved aligning the 16S rRNA gene sequences of the isolates with those of reference-type strains using the Clustal_W program (Larkin *et al.* 2007), with manual verification via EzEditor (Jeon *et al.* 2014). Phylogenetic trees were

Table 1. Summary of 22 isolates belonging to the phylum *Actinomycetota* and their taxonomic affiliations, isolation sources, and culture conditions

Phylum	Class	Order	Family	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation conditions
Actinomycetota	*Actinomycetia*	*Catenulisporales*	*Catenulisporaceae*	LS9-103	NNIBR2021641BA1680	*Catenulispora rubra*	99.4	Riverside sediment	1/10 R2A	3 days, 25°C
		Cellulomonadales	*Cellulomonadaceae*	23ND14S-41	NNIBR2023641BA2539	*Cellulomonas algicola*	99.9	Riverside sediment	MA	3 days, 25°C
			Jonesiaceae	23ND75W-61	NNIBR2023641BA2564	*Populibacterium corticicola*	99.3	Surface water	MA	3 days, 25°C
		Dermatophilales	*Intrasporangiaceae*	21SJ05W-0M23	NNIBR2021641BA1533	*Intrasporangium flavum*	98.7	Surface water	R2A	3 days, 20°C
		Dermatophilales	*Ornithinimicrobiaceae*	23ND49W-34	NNIBR2023641BA2550	*Ornithinimicrobium flavum*	99.7	Surface water	MA	3 days, 25°C
		Geodermatophilales	*Geodermatophilaceae*	HRW3-25	NNIBR2017301BA1	*Blastococcus aurantiacus*	99.8	Surface water	R2A	3 days, 25°C
				6LW2-11	NNIBR2022641BA2177	*Blastococcus endophyticus*	99.4	Surface water	R2A	3 days, 25°C
		Kineosporiales	*Kineosporiaceae*	22LW2-51	NNIBR2022641BA2283	*Kineosporia rhamnosa*	99.4	Surface water	R2A	3 days, 25°C
		Microbacteriales	*Microbacteriaceae*	23ND25W-67	NNIBR2023641BA615	*Arenivirga flava*	99.5	Surface water	ISP4	3 days, 20°C
				LW1-R25	NNIBR2022641BA232	*Cryobacterium breve*	99.3	Surface water	R2A	3 days, 20°C
		Micromonosporales	*Micromonosporaceae*	22LW2-48	NNIBR2022641BA2282	*Actinoplanes digitatis*	99.0	Surface water	R2A	3 days, 25°C
				23ND14S-8	NNIBR2023641BA2545	*Micromonospora globbae*	99.8	Riverside sediment	1/10TSA	2 days, 25°C
		Mycobacteriales	*Corynebacteriaceae*	SS-105	NNIBR2023641BA2542	*Corynebacterium variabile*	99.5	Insect intestines	1/10TSA	2 days, 25°C
			Gordoniaceae	SJ8	NNIBR2016301BA1	*Gordonia sputi*	99.9	Surface water	R2A	3 days, 25°C
			Nocardiaceae	21LS8-22	NNIBR2021641BA1247	*Nocardia stercoris*	99.3	Riverside sediment	R2A	3 days, 25°C
				23ND34S-94	NNIBR2023641BA2546	*Nocardia rayongensis*	99.7	Riverside sediment	1/10 R2A	2 days, 25°C
			Mycobacteriaceae	SJW1-30	NNIBR2017301BA9	*Mycobacterium gilvum*	99.3	Surface water	NA	3 days, 25°C
		Propionibacteriales	*Nocardioidaceae*	21LS6-111	NNIBR2021641BA147	*Nocardioides allogilvus*	99.4	Riverside sediment	1/10 R2A	3 days, 25°C
				23ND01W-24	NNIBR2023641BA2538	*Nocardioides lianchengensis*	100.0	Surface water	1/10TSA	2 days, 25°C
				23ND54S-044	NNIBR2023641BA2552	*Nocardioides kandeliae*	99.3	Riverside sediment	MA	3 days, 25°C
			Propionibacteriaceae	21SJ05S-R33	NNIBR2021641BA1532	*Aestuariimicrobium soli*	98.7	Surface water	R2A	3 days, 20°C
	Thermoleophilia	*Solirubrobacterales*	*Solirubrobacteraceae*	21SJ02S-54	NNIBR2021641BA1655	*Solirubrobacter pauli*	99.7	Riverside sediment	R2A	3 days, 20°C

constructed based on the aligned sequences using the neighbor-joining method (Saitou and Nei 1987) and the Kimura 2-parameter model (Kimura 1980) implemented in MEGA 7.0 software (Kumar *et al.* 2016). The robustness of the phylogenetic trees was tested through bootstrap analyses with 1,000 random re-samplings (Felsenstein 1985).

The colony morphologies of the bacterial strains were examined on agar plates using a magnifying glass after cultivation to the stationary phase. Transmission electron microscopy further examined cellular morphology and size (Talos L120C; ThermoFisher Scientific, Waltham, MA, USA). Gram staining was performed using a Gram-staining kit (BioMérieux, Marcy-I'Étoile, France). Additionally, biochemical characteristics were assessed with API 20NE galleries (BioMérieux) following the provided protocols.

3. RESULTS AND DISCUSSION

The 16S rRNA gene sequence analyses conducted on approximately 1,000 bacterial strains obtained in this study revealed the presence of novel species or species previously unreported in Korea. Specifically, 22 strains exhibited $\geq$98.7% 16S rRNA gene sequence similarities with unrecorded *Actinomycetota* species in Korea. These strains belonged to one phylum, two classes, ten orders, and eighteen genera. The detailed taxonomic composition and identification results of these species are summarized in Table 1. The phylogenetic tree of the bacterial strains assigned to the phylum *Actinomycetota* is shown in Figure 1. This figure depicts the phylogenetic relationships between the isolates and closely related species. Transmission electron microscopic images of the isolates are provided in Figure 2.

The identification of these 22 previously unrecorded *Actinomycetota* species enhances our understanding of microbial diversity in freshwater ecosystems. *Actinomycetota*, known for their role in nutrient cycling, organic matter decomposition, and maintenance of water quality, are critical components of these ecosystems. The discovery of these species suggests the presence of unique ecological niches within the freshwater environments of Korea, highlighting the importance of continued microbial exploration in underexplored habitats.

Actinomycetota are renowned for their production of bioactive secondary metabolites, including antibiotics, antifungals, and immunosuppressive agents. The newly identified species hold considerable promise for biotechnological applications. For instance, the genus *Streptomyces*, well-known for its antibiotic production, includes newly discovered strains that could potentially yield novel antimicrobial compounds. Additionally, species from the genera *Micromonospora* and *Nocardia* have been previously noted for their roles in bioremediation, suggesting that the newly identified species may contribute to sustainable environmental practices by degrading pollutants and improving water quality. Future research should focus on investigating the functional properties and ecological roles of these bacteria to fully realize their potential benefits.

In addition to the *Actinomycetota* species isolated from Korean freshwater environments, similar studies have been conducted in other parts of Asia, particularly in Japan and China. These studies provide valuable insights into the diversity and distribution of *Actinomycetota* across different freshwater ecosystems. For instance, a study conducted in Japan reported the isolation of novel *Actinomycetota* species from river sediments, highlighting the presence of unique microbial communities adapted to specific environmental conditions (Lipko and Belykh 2021). Similarly, research in China has revealed the presence of diverse *Actinomycetota* species in various freshwater habitats, including lakes and rivers, some of which have demonstrated significant potential for producing bioactive compounds (Chen *et al.* 2021; Wu *et al.* 2021). A comparison of the *Actinomycetota* species isolated from Korean freshwater with those from Japan and China reveals both similarities and distinct differences in the microbial communities. For example, while some genera such as *Streptomyces* and *Micromonospora* are commonly found across all three regions, species diversity may differ due to variations in environmental factors such as pH, temperature, and nutrient availability. Moreover, the unique geographic and climatic conditions of Korea may contribute to the presence of species not yet reported in Japan or China, underscoring the importance of exploring underexplored habitats to uncover novel microbial taxa. This comparison also emphasizes the importance of regional studies in understanding the global diversity of *Actinomycetota* and their potential applications. Continued exploration and characterization of *Actinomycetota* in different regions

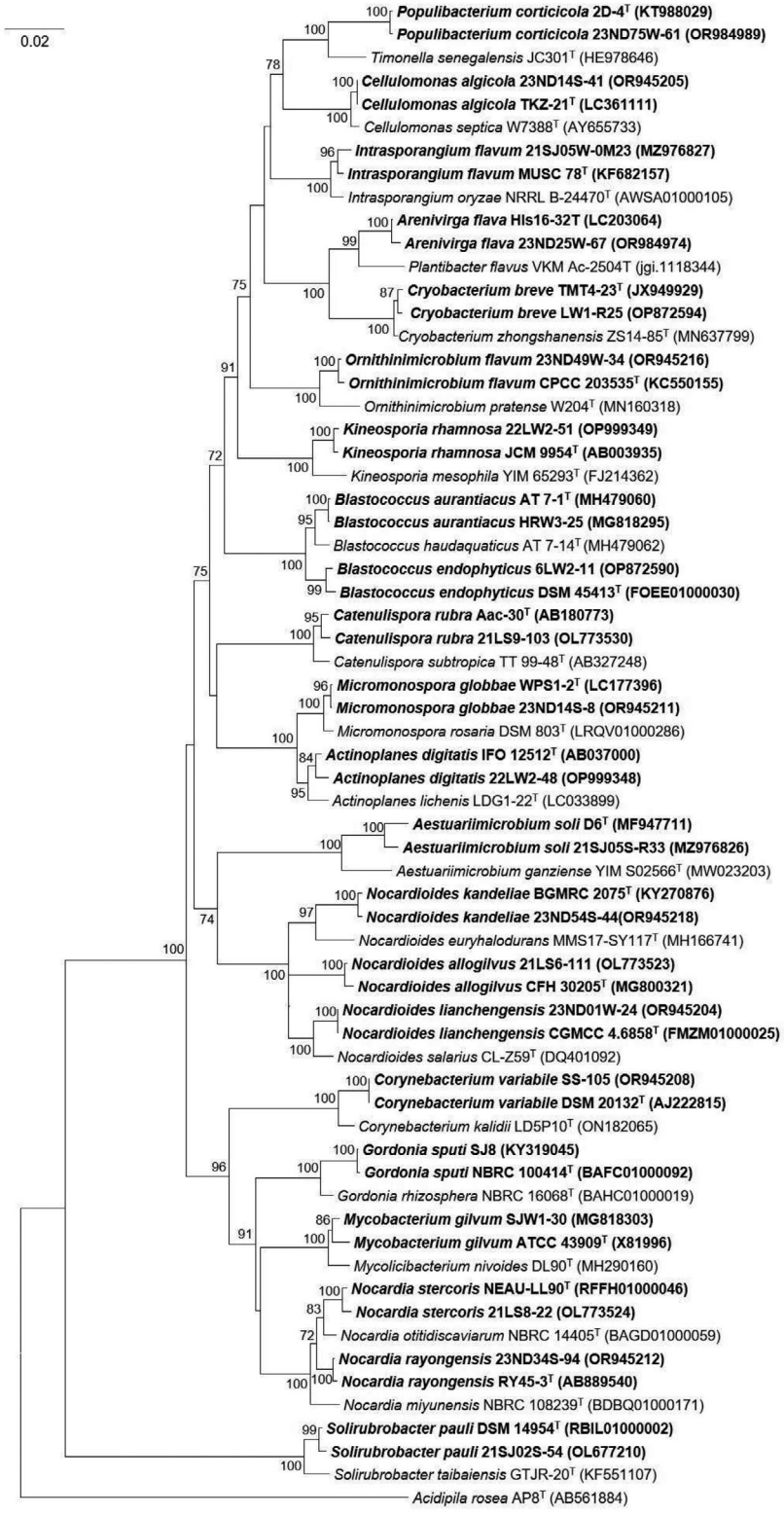

Fig. 1. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing relationships of 22 strains isolated in this study and their relatives in the phylum *Actinomycetota*. *Acidipila rosea* AP8^T (GenBank accession no. AB561884) was used as an outgroup. Bootstrap values (>70%) are shown at nodes. Bar, 0.02 substitutions per nucleotide position.

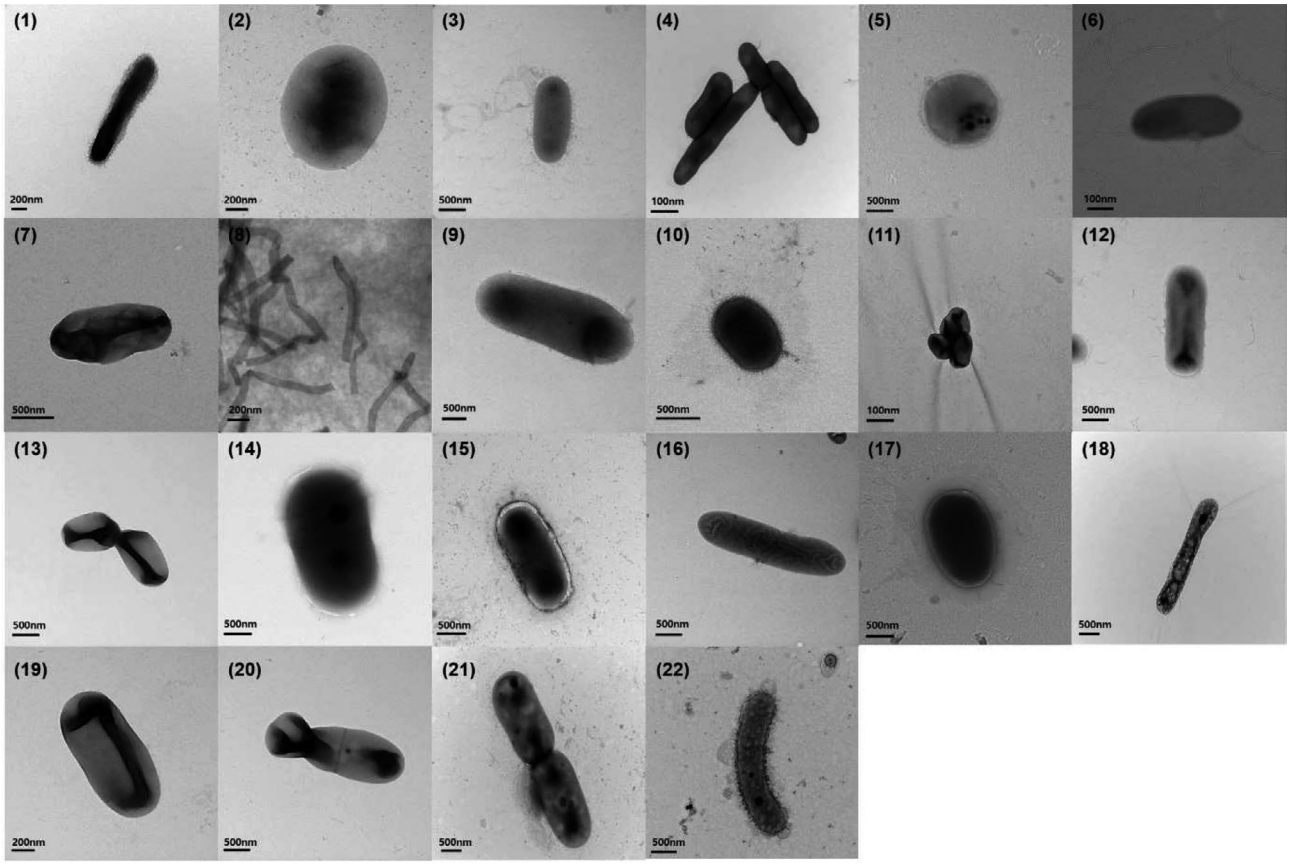

Fig. 2. Transmission electron micrographs of cells of strains isolated in this study. Scale bars are shown for each image. Strains: 1, LS9-103; 2, 23ND14S-41; 3, 23ND75W-61; 4, 21SJ05W-0M23; 5, 23ND49W-34; 6, HRW3-25; 7, 6LW2-11; 8, 22LW2-51; 9, 23ND25W-67; 10, LW1-R25; 11, 22LW2-48; 12, 23ND14S-8; 13, SS-105; 14, SJ8; 15, 21LS8-22; 16, 23ND34S-94; 17, SJW1-30; 18, 21LS6-111; 19, 23ND01W-24; 20, 23ND54S-044; 21, 21SJ05S-R33; 22, 21SJ02S-54.

are essential for uncovering new species that may hold promise for biotechnological applications, such as the development of new antibiotics or bioremediation agents.

3.1. Description of *Catenulispora rubra* LS9-103

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on 1/10 R2A are circular, translucent, substrate mycelium, and ivory after incubation for 3 days at 25°C. Positive for glucose fermentation, arginine dihydrolase, and urease, but nitrate reduction, indole production, esculin hydrolysis, gelatinase, and β-galactosidase. Phenylacetic acid is utilized but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate. Strain LS9-103 displays the highest 16S rRNA gene sequence similarity with *Catenulispora rubra* Aac-30^{T} (99.4%). Strain LS9-103 (=NNIBR2021641BA1680=FBCC-B8703) was isolated from riverside sediment collected in Gangneung-si, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OL773530.

3.2. Description of *Cellulomonas algicola* 23ND14S-41

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on MA are circular, raised, entire, and yellow after incubation for 3 days at 25°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase, and β-galactosidase, but nitrate reduction, indole production, arginine dihydrolase, and urease. D-Glucose, L-arabinose, D-mannose, D-mannitol,

N-acetyl-glucosamine, D-maltose, and potassium gluconate are utilized. Does not utilize capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 23ND14S-41 displays the highest 16S rRNA gene sequence similarity with *Cellulomonas algicola* TKZ-21^{T} (99.9%). Strain 23ND14S-41 (=NNIBR 2023641BA2539=FBCC-B15847) was isolated from riverside sediment collected in Hadong-gun, Gyeongsangnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945205.

3.3. Description of *Populibacterium corticicola* 23ND75W-61

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on MA are umbonate, and creamy-white after incubation for 3 days at 25°C. Positive for esculin hydrolysis, and *β*-galactosidas, but nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, and D-maltose are utilized. Does not utilize *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 23ND75W-61 displays the highest 16S rRNA gene sequence similarity with *Populibacterium corticicola* 2D-4^{T} (99.3%). Strain 23ND75W-61 (=NNIBR2023641BA2564=FBCC-B15808) was isolated from the surface water of a stream collected in Seosan-si, Chungcheongnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR984989.

3.4. Description of *Intrasporangium flavum* 21SJ05W-0M23

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on R2A are circular convex entire, and pale yellow after incubation for 3 days at 20°C. Positive for nitrate reduction, gelatinase, and *β*-galactosidas, but indole production, glucose fermentation, arginine dihydrolase, urease, and esculin hydrolysis. L-Arabinose, D-mannose, D-maltose, potassium gluconate, and adipic acid are utilized. Does not utilize D-glucose, D-mannitol, *N*-acetyl-glucosamine, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 21SJ05W-0M23 displays the highest 16S rRNA gene sequence similarity with *Intrasporangium flavum* MUSC 78^{T} (98.7%). Strain 21SJ05W-0M23 (=NNIBR2021641BA1533=FBCC-B8168) was isolated from surface water collected in Imsil-gun, Jeollabuk-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is MZ976827.

3.5. Description of *Ornithinimicrobium flavum* 23ND49W-34

Cells are Gram-stain-positive, non-flagellated, and oval-shaped. Colonies grown on MA are circular raised entire, and yellow after incubation for 3 days at 25°C. Positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and *β*-galactosidas. D-Maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate, and phenylacetic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and capric acid. Strain 23ND49W-34 displays the highest 16S rRNA gene sequence similarity with *Ornithinimicrobium flavum* CPCC 203535^{T} (99.7%). Strain 23ND49W-34 (=NNIBR2023641BA2550=FBCC-B15836) was isolated from surface water collected in Namhae-gun, Gyeongsangnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945216.

3.6. Description of *Blastococcus aurantiacus* HRW3-25

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on R2A are circular, convex, smooth, and orange after incubation for 3 days at 25°C. Positive for nitrate reduction, indole production, esculin hydrolysis, and *β*-galactosidase, but negative for glucose fermentation, arginine dihydrolase, urease, and gelatinas. Does not all utilize substrates: D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain HRW3-25 displays the highest 16S rRNA gene sequence similarity with *Blastococcus aurantiacus* AT 7-1^{T} (99.8%). Strain HRW3-25 (=NNIBR2017301BA1=FBCC-B3542) was isolated from surface water collected in Hanam-si, Gyeonggi-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is MG818295.

3.7. Description of *Blastococcus endophyticus* 6LW2-11

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on R2A are circular, convex, smooth, and cream after incubation for 3 days at 25°C. Positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidas. D-Glucose and D-mannitol are utilized. Does not utilize L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 6LW2-11 displays the highest 16S rRNA gene sequence similarity with *Blastococcus endophyticus* DSM 45413^T (99.4%). Strain 6LW2-11 (=NNIBR2022641BA2177=FBCC-B11910) was isolated from surface water collected in Yangyang-gun, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OP872590.

3.8. Description of *Kineosporia rhamnosa* 22LW2-51

Cells are Gram-stain-negative, non-flagellated, and rod-shaped. Colonies grown on R2A are wrinkle, brittle, umbonate, substrate mycelium developed, and light orange after incubation for 3 days at 25°C. Positive for esculin hydrolysis, gelatinase, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid are utilized. Does not utilize capric acid, trisodium citrate, and phenylacetic acid. Strain 22LW2-51 displays the highest 16S rRNA gene sequence similarity with *Kineosporia rhamnosa* JCM 9954^T (99.4%). Strain 22LW2-51 (=NNIBR 2022641BA2283=FBCC-B12124) was isolated from surface water collected in Yangyang-gun, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OP999349.

3.9. Description of *Arenivirga flava* 23ND25W-67

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on ISP4 are punctiform, flat, and white after incubation for 3 days at 20°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinas. Does not all utilize substrates: D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 23ND25W-67 displays the highest 16S rRNA gene sequence similarity with *Arenivirga flava* HIs16-32^T (99.5%). Strain 23ND25W-67 (=NNIBR 2023641BA615=FBCC-B13684) was isolated from surface water collected in Gumi-si, Gyeongsangbuk-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR984974.

3.10. Description of *Cryobacterium breve* LW1-R25

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on R2A are circular, convex, smooth, and yellow after incubation for 3 days at 20°C. Positive for glucose fermentation, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid, and phenylacetic acid are utilized. Does not utilize capric acid, adipic acid, trisodium citrate. Strain LW1-R25 displays the highest 16S rRNA gene sequence similarity with *Cryobacterium breve* TMT4-23^T (99.3%). Strain LW1-R25 (=NNIBR 2022641BA232=FBCC-B9166) was isolated from surface water collected in Goseong-gun, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OP872594.

3.11. Description of *Actinoplanes digitatis* 22LW2-48

Cells are Gram-stain-negative, non-flagellated, and rod-shaped. Colonies grown on R2A are small, brittle, opaque, substrate mycelium developed, and light orange after incubation for 3 days at 25°C. Positive for esculin hydrolysis, gelatinase, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, and urease.

D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, adipic acid, and malic acid are utilized. Does not utilize capric acid, trisodium citrate, and phenylacetic acid. Strain 22LW2-48 displays the highest 16S rRNA gene sequence similarity with *Actinoplanes digitatis* IFO 12512^T (99.0%). Strain 22LW2-48 (=NNIBR 2022641BA2282=FBCC-B12123) was isolated from surface water collected in Yangyang-gun, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OP999348.

3.12. Description of *Micromonospora globbae* 23ND14S-8

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on 1/10 TSA are circular raised entire, and brown after incubation for 2 days at 25°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinas. L-Arabinose, D-mannitol, D-maltose, potassium gluconate, adipic acid, malic acid, and trisodium citrate are utilized. Does not utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, capric acid, and phenylacetic acid. Strain 23ND14S-8 displays the highest 16S rRNA gene sequence similarity with *Micromonospora globbae* WPS1-2^T (99.8%). Strain 23ND14S-8 (=NNIBR2023641BA2545=FBCC-B15841) was isolated from riverside sediment collected in Hadong-gun, Gyeongsangnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945211.

3.13. Description of *Corynebacterium variabile* SS-105

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on 1/10 TSA are circular raised entire, and white after incubation for 2 days at 25°C. Positive for glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, and gelatinase. Utilize all substrates: D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain SS-105 displays the highest 16S rRNA gene sequence similarity with *Corynebacterium variabile* DSM 20132^T (99.5%). Strain SS-105 (=NNIBR2023641BA2542=FBCC-B15844) was isolated from insect intestine collected from fresh water in Jangseong-gun, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945208.

3.14. Description of *Gordonia sputi* SJ8

Cells are Gram-stain-negative, non-flagellated, and rod-shaped. Colonies grown on R2A are circular, viscous, convex with entire edge, and mily orange after incubation for 3 days at 25°C. Positive for nitrate reduction, urease, and esculin hydrolysis but negative for indole production, glucose fermentation, arginine dihydrolase, gelatinase, and β-galactosidase. D-Glucose, D-mannose, malic acid, and phenylacetic acid are utilized. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, and trisodium citrate. Strain SJ8 displays the highest 16S rRNA gene sequence similarity with *Gordonia sputi* NBRC 100414^T (99.9%). Strain SJ8 (=NNIBR2016301BA1=FBCC-B3259) was isolated from surface water collected in Gwangyang-si, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is KY319045.

3.15. Description of *Nocardia stercoris* 21LS8-22

Cells are Gram-stain-negative, non-flagellated, and rod-shaped. Colonies grown on R2A are circular, substrate mycelium, aerial mycelium, and yellowish white after incubation for 3 days at 25°C. Positive for nitrate reduction and urease, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β-galactosidas. Phenylacetic acid is utilized but does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate. Strain 21LS8-22 displays the highest 16S rRNA gene sequence similarity with *Nocardia stercoris* NEAU-LL90^T (99.3%). Strain 21LS8-22 (=NNIBR 2021641BA1247=FBCC-B7675) was isolated from riverside sediment collected in Goseong-gun, Gangwon-do, Korea. The GenBank accession number of the

16S rRNA gene sequence is OL773524.

3.16. Description of *Nocardia rayongensis* 23ND34S-94

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on 1/10 R2A are circular, raised, entire, and white after incubation for 2 days at 25°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. L-Arabinose, malic acid, and trisodium citrate are utilized. Does not utilize D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, and phenylacetic acid. Strain 23ND34S-94 displays the highest 16S rRNA gene sequence similarity with *Nocardia rayongensis* RY45-3^T (99.7%). Strain 23ND34S-94 (=NNIBR2023641BA2546=FBCC-B15840) was isolated from riverside sediment collected in Namhae-gun, Gyeongsangnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945212.

3.17. Description of *Mycobacterium gilvum* SJW1-30

Cells are Gram-stain-positive, non-flagellated, and short rod-shaped. Colonies grown on NA are circular, convex, and yellow after incubation for 3 days at 25°C. Positive for nitrate reduction and urease, but negative for indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β-galactosidase. D-Mannitol and malic acid are utilized. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain SJW1-30 displays the highest 16S rRNA gene sequence similarity with *Mycobacterium gilvum* ATCC 43909^T (99.3%). Strain SJW1-30 (=NNIBR2017301BA9=FBCC-B3425) was isolated from surface water collected in Gwangyang-si, Jeollanam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is MG818303.

3.18. Description of *Nocardioides allogilvus* 21LS6-111

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on 1/10 R2A are circular, smooth, opaque, and pale yellow after incubation for 3 days at 25°C. Positive for urease, esculin hydrolysis, gelatinase, and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, and arginine dihydrolase. D-Glucose and phenylacetic acid are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, and trisodium citrate. Strain 21LS6-111 displays the highest 16S rRNA gene sequence similarity with *Nocardioides allogilvus* CFH 30205^T (99.4%). Strain 21LS6-111 (=NNIBR2021641BA147=FBCC-B6334) was isolated from riverside sediment collected in Goseong-gun, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OL773523.

3.19. Description of *Nocardioides lianchengensis* 23ND01W-24

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on 1/10 TSA are circular, raised, entire, and white after incubation for 2 days at 25°C. Positive for glucose fermentation, esculin hydrolysis, and β-galactosidase, but negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. D-Glucose, L-arabinose, D-mannitol, potassium gluconate, and malic acid are utilized. Does not utilize D-mannose, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain 23ND01W-24 displays the highest 16S rRNA gene sequence similarity with *Nocardioides lianchengensis* CGMCC 4.6858^T (100.0%). Strain 23ND01W-24 (=NNIBR2023641BA2538=FBCC-B15848) was isolated from surface water collected in Hamyang-gun, Gyeongsangnam-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945204.

3.20. Description of *Nocardioides kandeliae* 23ND54S-44

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on MA are circular, raised, entire, and white after incubation for 3 days at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β-galactosi-

dase. D-Glucose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, and adipic acid are utilized. Does not utilize L-arabinose, D-mannose, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 23ND54S-44 displays the highest 16S rRNA gene sequence similarity with *Nocardioides kandeliae* BGMRC 2075^T (99.3%). Strain 23ND54S-44 (=NNIBR2023641BA2552=FBCC-B15834) was isolated from riverside sediment collected in Samcheok-si, Gangwon-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OR945218.

3.21. Description of *Aestuariimicrobium soli* 21SJ05S-R33

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on R2A are convex with entire edge, and ivory after incubation for 3 days at 20°C. Positive for nitrate reduction, esculin hydrolysis, and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. Does not utilize all substrates: D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 21SJ05S-R33 displays the highest 16S rRNA gene sequence similarity with *Aestuariimicrobium soli* D6^T (98.7%). Strain 21SJ05S-R33 (=NNIBR2021641BA1532=FBCC-B8167) was isolated from surface water collected in Imsil-gun, Jeollabuk-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is MZ976826.

3.22. Description of *Solirubrobacter pauli* 21SJ02S-54

Cells are Gram-stain-positive, non-flagellated, and rod-shaped. Colonies grown on R2A are circular, convex, smooth, and white after incubation for 3 days at 20°C. Positive for esculin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β-galactosidase. Does not utilize all substrates: D-Glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain 21SJ02S-54 displays the highest 16S rRNA gene sequence similarity with *Solirubrobacter pauli* DSM 14954^T (99.7%). Strain 21SJ02S-54 (=NNIBR2021641BA1655=FBCC-B8600) was isolated from riverside sediment collected in Imsil-gun, Jeollabuk-do, Korea. The GenBank accession number of the 16S rRNA gene sequence is OL677210.

4. CONCLUSION

This study successfully identified and characterized 22 previously unrecorded bacterial species from the phylum *Actinomycetota* in Korean freshwater environments. These findings significantly expand the known diversity of *Actinomycetota* and underscore the importance of exploring underexplored habitats for discovering novel microbial taxa. The newly identified species hold considerable promise for various biotechnological applications, such as the development of new antimicrobial agents and bioremediation strategies. Future research should focus on investigating the functional properties and ecological roles of these bacteria to fully realize their potential benefits.

CRediT authorship contribution statement

SY Lee: Investigation, Data curation, Writing, Funding acquisition, Writing-Reviewing and editing. **J Goh:** Supervision. **A Choi:** Project administration, Conceptualization, Writing, Funding acquisition, Writing-Reviewing and editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Nakdonggang National Institute of Biological Resources (NNIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NNIBR20241103).

REFERENCES

Bae KS, MS Kim, JH Lee, JW Kang, DI Kim, JH Lee and CN Seong. 2016. Korean indigenous bacterial species with valid names belonging to the phylum *Actinobacteria*. J. Microbiol. 54:789–795. https://doi.org/10.1007/s12275-016-6446-4

Behera S and S Das. 2023. Potential and prospects of *Actinobacteria* in the bioremediation of environmental pollutants: Cellular mechanisms and genetic regulations. Microbiol. Res. 273: 127399. https://doi.org/10.1016/j.micres.2023.127399

Bérdy J. 2005. Bioactive microbial metabolites. J. Antibiot. 58:1–26. https://doi.org/10.1038/ja.2005.1

Blin K, S Shaw, SA Kautsar, MH Medema and T Weber. 2021. The antiSMASH database version 3: increased taxonomic coverage and new query features for modular enzymes. Nucleic Acids Res. 49:D639–D643. https://doi.org/10.1093/nar/gkaa978

Böckelmann U, W Manz, TR Neu and U Szewzyk. 2000. Characterization of the microbial community of lotic organic aggregates ('river snow') in the Elbe River of Germany by cultivation and molecular methods. FEMS Microbiol. Ecol. 33:157–170. https://doi.org/10.1016/S0168-6496(00)00056-8

Bruna P, K Núñez-Montero, MJ Contreras, K Leal, M García, M Abanto and L Barrientos. 2024. Biosynthetic gene clusters with biotechnological applications in novel Antarctic isolates from *Actinomycetota*. Appl. Microbiol. Biotechnol. 108:325. https://doi.org/10.1007/s00253-024-13154-x

Chen J, L Xu, Y Zhou and B Han. 2021. Natural products from *Actinomycetes* associated with marine organisms. Mar. Drugs 19:629. https://doi.org/10.3390/md19110629

Comba S, S Menendez-Bravo, A Arabolaza and H Gramajo. 2013. Identification and physiological characterization of phosphatidic acid phosphatase enzymes involved in triacylglycerol biosynthesis in *Streptomyces coelicolor*. Microb. Cell Fact. 12:9. https://doi.org/10.1186/1475-2859-12-9

Donald L, A Pipite, R Subramani, J Owen, RA Keyzers and T Taufa. 2022. *Streptomyces*: Still the biggest producer of new natural secondary metabolites, a current perspective. Microbiol. Res. 13:418–465. https://doi.org/10.3390/microbiolres13030031

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Ferreira V, A Elosegi, SD Tiegs, D von Schiller and R Young. 2020. Organic matter decomposition and ecosystem metabolism as tools to assess the functional integrity of streams and rivers — A systematic review. Water 12:3523. https://doi.org/10.3390/w12123523

Gao B and RS Gupta. 2012. Phylogenetic framework and molecular signatures for the main clades of the phylum *Actinobacteria*. Microbiol. Mol. Biol. Rev. 76:66–112. https://doi.org/10.1128/mmbr.05011-11

Goodfellow M and ST Williams. 1983. Ecology of *Actinomycetes*. Annu. Rev. Microbiol. 37:189–216. https://doi.org/10.1146/annurev.mi.37.100183.001201

Heo CS, JS Kang, JH Kwon, CV Anh and HJ Shin. 2023. Pyrrole-containing alkaloids from a marine-derived actinobacterium *Streptomyces zhaozhouensis* and their antimicrobial and cytotoxic activities. Mar. Drugs 21:167. https://doi.org/10.3390/md21030167

Jeon YS, K Lee, SC Park, BS Kim, YJ Cho, SM Ha and J Chun. 2014. EzEditor: a versatile sequence alignment editor for both rRNA-and protein-coding genes. Int. J. Syst. Evol. Microbiol. 64:689–691. https://doi.org/10.1099/ijs.0.059360-0

Kim S, MR Islam, I Kang and JC Cho. 2021. Cultivation of dominant freshwater bacterioplankton lineages using a high-throughput dilution-to-extinction culturing approach over a 1-year period. Front. Microbiol. 12:700637. https://doi.org/10.3389/fmicb.2021.700637

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120. https://doi.org/10.1007/BF01731581

Kumar S, G Stecher and K Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Larkin MA, G Blackshields, NP Brown, R Chenna, PA McGettigan, H McWilliam, F Valentin, IM Wallace, A Wilm, R Lopez, JD Thompson, TJ Gibson and DG Higgins. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948. https://doi.org/10.1093/bioinformatics/btm404

Lee HJ, SI Han and KS Whang. 2016. Phylogenetic characteristics of actinobacterial population in bamboo (*Sasa borealis*) soil. Korean J. Microbiol. 52:59–64. https://doi.org/10.7845/kjm.2016.6006

Lipko IA and OI Belykh. 2021. Environmental features of freshwater planktonic *Actinobacteria*. Contemp. Probl. Ecol. 14:158–170. https://doi.org/10.1134/S1995425521020074

Ngamcharungchit C, N Chaimusik, W Panbangred, J Euanorasetr and B Intra. 2023. Bioactive metabolites from terrestrial and marine *Actinomycetes*. Molecules 28:5915. https://doi.org/10.3390/molecules28155915

Oren A and GM Garrity. 2021. Valid publication of the names of forty-two phyla of prokaryotes. Int. J. Syst. Evol. Microbiol. 71:005056. https://doi.org/10.1099/ijsem.0.005056

Saitou N and M Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4:406–425. https://doi.org/10.1093/oxfordjournals.molbev.a040454

Servin JA, CW Herbold, RG Skophammer and JA Lake. 2008. Evidence excluding the root of the tree of life from the actinobacteria. Mol. Biol. Evol. 25:1–4. https://doi.org/10.1093/molbev/msm249

Slemc L, Š Pikl, H Petković and M Avbelj. 2021. Molecular biology methods in *Streptomyces rimosus*, a producer of oxytetracycline. pp. 303–330. In: Antimicrobial Therapies (Barreiro C and JL Barredo, eds.). Methods in Molecular Biology. Vol. 2296. Humana. New York, USA. https://doi.org/10.1007/978-1-0716-1358-0_18

Weisburg WG, SM Barns, DA Pelletier and DJ Lane. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. 173:697–703. https://doi.org/10.1128/jb.173.2.697-703.1991

Wu J, Z Peng, TW Guan, H Yang and X Tian. 2021. Diversity of actinobacteria in sediments of Qaidam Lake and Qinghai Lake, China. Arch. Microbiol. 203:2875–2885. https://doi.org/10.1007/s00203-021-02277-8

Yoon SH, SM Ha, S Kwon, J Lim, Y Kim, H Seo and J Chun. 2017. Introducing EzBioCloud: A taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67:1613–1617. https://doi.org/10.1099/ijsem.0.001755

