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Report of 14 unrecorded bacterial species in Korea belonging to the phyla *Bacteroidota*, *Acidobacteriota* and *Verrucomicrobiota*, excavated during the survey in 2023

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In this study, a total of 14 bacterial strains belonging to the phyla *Bacteroidota*, *Acidobacteriota* and *Verrucomicrobiota* isolated in 2023 are reported as unrecorded species in Korea. Among 14 bacterial strains, 12 strains were assigned to the phylum *Bacteroidota*, including 5 species belonging to the family *Flavobacteriaceae*, 3 species to the family *Weeksellaceae*, 2 species to the family *Cyclobacteriaceae* and 1 species each to the families *Prolixibacteraceae* and *Sphingobacteriaceae*. At genus level, *Flavobacterium* (5 species) was the most abundant genus, which was then followed by *Chryseobacterium* (3 species) and *Algoriphagus* (2 species). Single species were obtained for the genera *Sunxiuqinia* and *Mucilaginibacter*. In addition, one isolate assigned to the phylum *Acidobacteriota* belonged to the genus *Edaphobacter* of the family *Acidobacteriaceae*, and the other assigned to the phylum *Verrucomicrobiota* belonged to the genus *Verrucomicrobium* of the family *Verrucomicrobiaceae*.

Keywords: *Acidobacteriota*, *Bacteroidota*, *Flavobacterium*, unrecorded species, *Verrucomicrobiota*

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Introduction

The phyla *Bacteroidota*, *Acidobacteriota* and *Verrucomicrobiota* share several common characteristics. They are all Gram-stain-negative bacteria and exhibit diverse cellular morphologies, including rod-shaped, coccoid, and filamentous forms. These bacteria can be aerobic, facultative anaerobic, or obligate anaerobic, and they do not form endospores. They are widely distributed across various environments, including soil, aquatic ecosystems and host-associated microbiomes, and play significant roles in ecological processes such as carbon and nitrogen cycling, nutrient degradation, and host interactions.

Bacteroidota are primarily found in the gut and skin of animals, as well as in marine and terrestrial environments. They are known for their role in nutrient digestion, calorie absorption, immune regulation, metabolic processes, and the gut-brain axis. These bacteria contribute significantly to host-microbiome interactions, particularly in the breakdown of complex carbohydrates (Krieg *et al*., 2010;

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Thomas *et al*., 2011; Wang *et al*., 2016).

Acidobacteriota are predominantly soil-dwelling bacteria, and are particularly abundant in acidic environments. They have a remarkable tolerance to low pH and play a crucial role in carbon and nitrogen cycling within soil ecosystems. Some species are capable of metabolizing complex carbohydrates, thus promoting soil health and plant growth (Thrash and Coates, 2010; Dedysh and Sinninghe Damsté, 2018; Kalam *et al*., 2020).

Verrucomicrobiota are found in a wide range of habitats, including soil, freshwater, marine environments, and extreme conditions such as hot springs. They contribute to carbon cycling by degrading complex carbohydrates, and some species are even involved in methane production. Notably, *Akkermansia muciniphila*, a representative species of this phylum, plays a key role in gut health by degrading mucin, an essential glycoprotein in mucus, thereby influencing host metabolism and immune function (Hedlund, 2010; Derrien *et al*., 2017; Orellana *et al*., 2021).

In a series of extensive surveys on the prokaryotic diversity in Korea in the year 2023, 14 novel strains belonging the phyla *Bacteroidota*, *Acidobacteriota* and *Verrucomicrobiota* were isolated from various environmental sources, and their taxonomic properties were analyzed. As a result, a total of 14 unrecorded species of Korea were identified, and their taxonomic properties are presented.

Materials and Methods

The strains were isolated from various environmental samples such as soil, mudflat, wetland, stream, agricultural soil, and mud (Table 1). The samples were processed or treated separately, diluted and spread onto diverse culture media, namely R2A, marine agar (MA) or peptone-tryptone-yeast extracts-glucose (PTYG) agar, and incubated at 25−37°C for 2−5 days. The designated strain codes, sources of isolation, culture media, and incubation conditions are provided in the descriptions and Table 1. All strains were purified as single colonies and stored in 10−20% glycerol suspension at −80°C as well as lyophilized ampoules.

Colony characteristics of the isolates were observed on the same agar media for cultivation. Cellular morphology and cell size were examined using either a transmission electron microscope or a scanning electron microscope. Gram staining was performed using standard procedures (Bartholomew and Mittwer, 1952). Biochemical characteristics were tested by using API 20NE galleries (bio Mérieux) according to the manufacturer's instructions.

The extraction of genomic DNA, PCR amplification of 16S rRNA gene and sequencing were performed using the procedures as described previously (Weisburg *et al*., 1991). The EzBioCloud database (Yoon *et al*., 2017) was

Table 1. Taxonomic affiliations of isolated strains belong to the phyla *Bacteroidota*, *Acidobacteriota* and *Verrucomicrobiota*

Affiliation	Strain ID	NIBR[a] ID	Identification	Similarity (%)[b]	Isolation source	Medium[c]	Culture conditions (°C, days)
Acidobacteriota	MA-22	NIBRBAC000510756	*Edaphobacter modestus*	98.94	Soil	R2A	25°C, 3 d
Bacteroidota	MMS23-Q5	NIBRBAC000510827	*Algoriphagus roseus*	99.09	Soil	R2A	30°C, 3 d
	JPR4	NIBRBAC000510805	*Algoriphagus kandeliae*	99.93	Mudflat	MA	28°C, 3 d
	AN795	NIBRBAC000510616	*Flavobacterium amnicola*	100.00	Wetland	R2A	28°C, 3 d
	CA1-11	NIBRBAC000510743	*Flavobacterium johnsoniae* subsp. *johnsoniae*	99.86	Soil	R2A	25°C, 3 d
	DL8	NIBRBAC000510651	*Flavobacterium ajazii*	99.16	Stream	R2A	25–30°C, 4 d
	FW4	NIBRBAC000510789	*Flavobacterium hercynium*	98.95	Agricultural soil	PTYG	28°C, 4 d
	MMS23-HJ70	NIBRBAC000510812	*Flavobacterium zaozhuangense*	99.37	Soil	R2A	30°C, 3 d
	CAU 1723	NIBRBAC000510780	*Sunxiuqinia elliptica*	99.93	Mud	MA	30°C, 3 d
	AR1	NIBRBAC000510794	*Mucilaginibacter rubeus*	100.00	Agricultural soil	R2A	28°C, 3 d
	ST11	NIBRBAC000510744	*Chryseobacterium polytrichastri*	99.24	Soil	R2A	25°C, 3 d
	WK20	NIBRBAC000510653	*Chryseobacterium aahli*	99.22	Soil	R2A	30°C, 2 d
	NW27	NIBRBAC000510634	*Chryseobacterium piscicola*	99.10	Stream	R2A	30°C, 3 d
Verrucomicrobiota	JDC8	NIBRBAC000510582	*Verrucomicrobium spinosum*	99.86	Soil	R2A	30°C, 2 d

[a]National Institute of Biological Resources.
[b]Sequence similarity based on 16S rRNA gene.
[c]R2A, Reasoner's 2A agar; MA, marine agar; PTYG, peptone-tryptone-yeast extract-glucose agar.

used for search of closest species, and their sequence were aligned using EzEditor2 (Jeon *et al*., 2014). Phylogenetic trees were generated by Jukes-Cantor distance mode (Jukes and Cantor, 1969) and neighbor-joining method (Saitou and Nei, 1987) using MEGA 7.0 (Kumar *et al*., 2016). The phylogenetic trees were evaluated using the bootstrap analysis (Felsenstein, 1985) based on 1,000 resampled datasets.

Results and Discussion

A total of 14 bacterial strains belonging to the phyla *Bacteroidota* (12 strains), *Acidobacteriota* (1 strain), and *Verrucomicrobiota* (1 strain) were isolated (Table 1). These strains represented 14 unrecorded species in Korea, belonging to 7 genera in 7 families. The 12 strains assigned to the phylum *Bacteroidota*, included 5 species belonging to the family *Flavobacteriaceae*, 2 species each to the families *Cyclobacteriaceae* and *Weeksellaceae*, and 1 species each to the families *Prolixibacteraceae* and *Sphingobacteriaceae*. At genus level, *Flavobacterium* (5 species) was the most abundant genus, which was then followed by *Chryseobacterium* (3 species) and *Algoriphagus* (2 species). Single species were obtained for the *Sunxiuqinia* and *Mucilaginibacter*. Among the remaining strains, one strain assigned to *Acidobacteriota* belonged to the genus *Edaphobacter* of the family *Acidobacteriaceae*, and the other assigned to *Verrucomicrobiota* belonged to the genus *Verrucomicrobium* of the family *Verrucomicrobiaceae*. The electron microscopic images of the isolates are provided in Fig. 1, and the phylogenetic relationship between the isolates and closely related species are presented in Figs. 2, 3 and 4. The detailed descriptions of each unrecorded species are provided.

Description of *Edaphobacter modestus* MA-22

Cells are Gram-stain-negative and rod-shaped. Motile by flagella. Colonies are circular, slightly convex, glistening and cream orange-colored after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis and β-galactosidase, and weakly positive for nitrate reduction, but negative for indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. 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 are not utilized as sole carbon sources. Strain MA-22 (=NIBRBAC000510756) was isolated from a soil sampled at Inje-gun, Gangwon-do, Republic of Korea (37°57′49.1″ N 128°19′53.7″E). The GenBank accession number for the 16S rRNA gene sequence of strain MA-22 is OR342862.

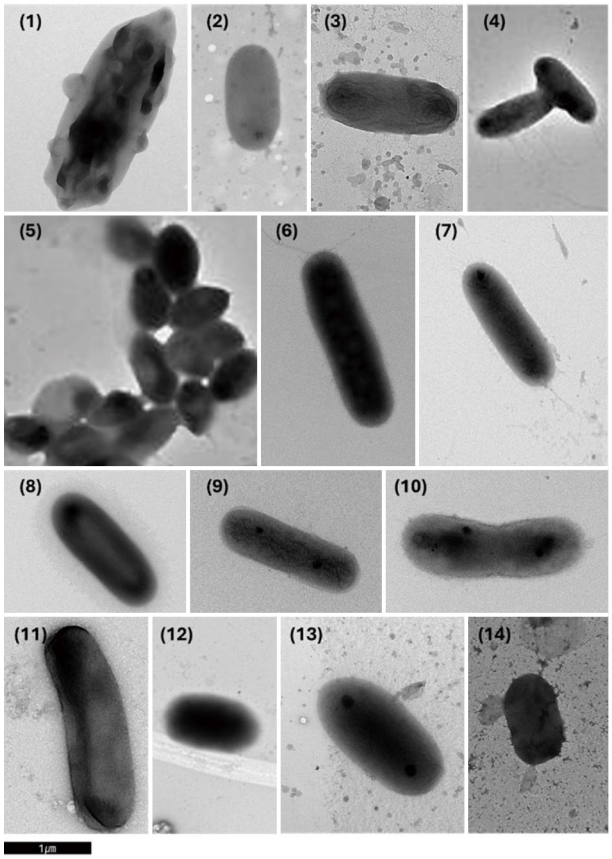


Fig. 1. Transmission electron or scanning electron micrographs of cells of the strains isolated in the study. Strain: 1, MA-22; 2, MMS23-Q5; 3, JPR4; 4, AN795; 5, CA1-11; 6, DL8; 7, FW4; 8, MMS23-HJ70; 9, CAU 1723; 10, AR1; 11, ST11; 12, WK20; 13, NW27; 14, JDC8. Scale bar, 1 μm.

Description of *Algoriphagus roseus* MMS23-Q5

Cells are Gram-stain-negative and ovoid-shaped. Colonies are circular, flat and pink-colored after incubation for 3 days on R2A at 30°C. Positive for esculin hydrolysis, gelatin hydrolysis 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, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized as sole carbon sources. Strain MMS23-Q5 (=NIBR BAC000510827) was isolated from a soil sampled at Heukseok-dong, Seo-gu, Daejeon, Republic of Korea (36° 16′15″N 127°21′21″E). The GenBank accession number for the 16S rRNA gene sequence of strain MMS23-Q5 is OR789442.

Description of *Algoriphagus kandeliae* JPR4

Cells are Gram-stain-negative and short rod-shaped. Colonies are circular, entire convex, smooth and red-color-

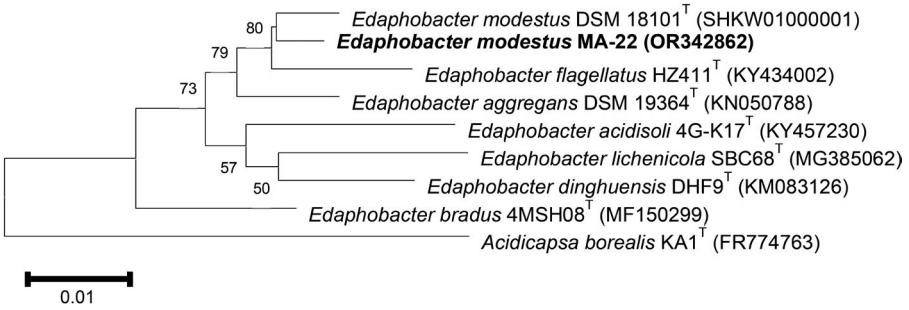


Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the family *Acidobacteriaceae* of the phylum *Acidobacteriota*. Bootstrap values (>50%) are shown at branching points. *Acidicapsa borealis* KA1^T used as an outgroup. Scale bar, 0.01 substitutions per nucleotide.

ed after incubation for 3 days on Marine agar at 28°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. 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 are not utilized as sole carbon sources. Strain JPR4 (=NIBRBAC000510805) was isolated from a mudflat sampled at Samsan-myeon, Ganghwa-gun, Incheon, Republic of Korea (37°70′95″N 126°30′99″E). The GenBank accession number for the 16S rRNA gene sequence of strain JPR4 is OR751828.

Description of *Flavobacterium amnicola* AN795

Cells are Gram-stain-negative and rod-shaped. Colonies are round, entire, convex and yellow-colored after incubation for 3 days on R2A at 28°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatin hydrolysis, β-galactosidase and cytochrome oxidase. 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 are not utilized as sole carbon sources. Strain AN795 (=NIBR BAC000510616) was isolated from a wetland sampled at Dogi-dong, Anseong-si, Gyeonggi-do, Republic of Korea (37°01′02.6″N 127°15′48.9″E). The GenBank accession number for the 16S rRNA gene sequence of strain AN795 is OR527152.

Description of *Flavobacterium johnsoniae* subsp. *johnsoniae* CA1-11

Cells are Gram-stain-negative and rod-shaped. Colonies are circular, slightly convex, glistening and yellow-colored after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis, gelatin hydrolysis 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, adipic acid, malic acid and trisodium citrate are utilized as sole carbon sources, but not potassium gluconate, capric acid and phenylacetic acid. Strain CA1-11 (=NIBRBAC000510743) was isolated from a soil sampled at Cheonan-si, Chungcheongnam-do, Republic of Korea (36°48′58.3″N 127°07′17.4″E). The GenBank accession number for the 16S rRNA gene sequence of strain CA1-11 is OR346149.

Description of *Flavobacterium ajazi* DL8

Cells are Gram-stain-negative and short rod-shaped. Colonies are circular, convex, opaque and bright yellow-colored after incubation for 4 days on R2A at 25–30°C. Positive for nitrate reduction, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, L-arabinose, D-mannose and D-maltose are utilized as sole carbon sources, but not D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain DL8 (=NIBRBAC000510651) was isolated from a stream sampled at Gongneungcheon, Neungan-ro, Jori-eup, Paju-si, Gyeonggi-do, Republic of Korea (37°44′ 29.6″N 126°47′35.2″E). The GenBank accession number for the 16S rRNA gene sequence of strain DL8 is OR251 346.

Description of *Flavobacterium hercynium* FW4

Cells are Gram-stain-negative and short rod-shaped. Colonies are circular, undulate, convex, smooth and white-colored after incubation for 4 days on PTYG agar at 28°C. Positive for nitrate reduction, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, malic acid and

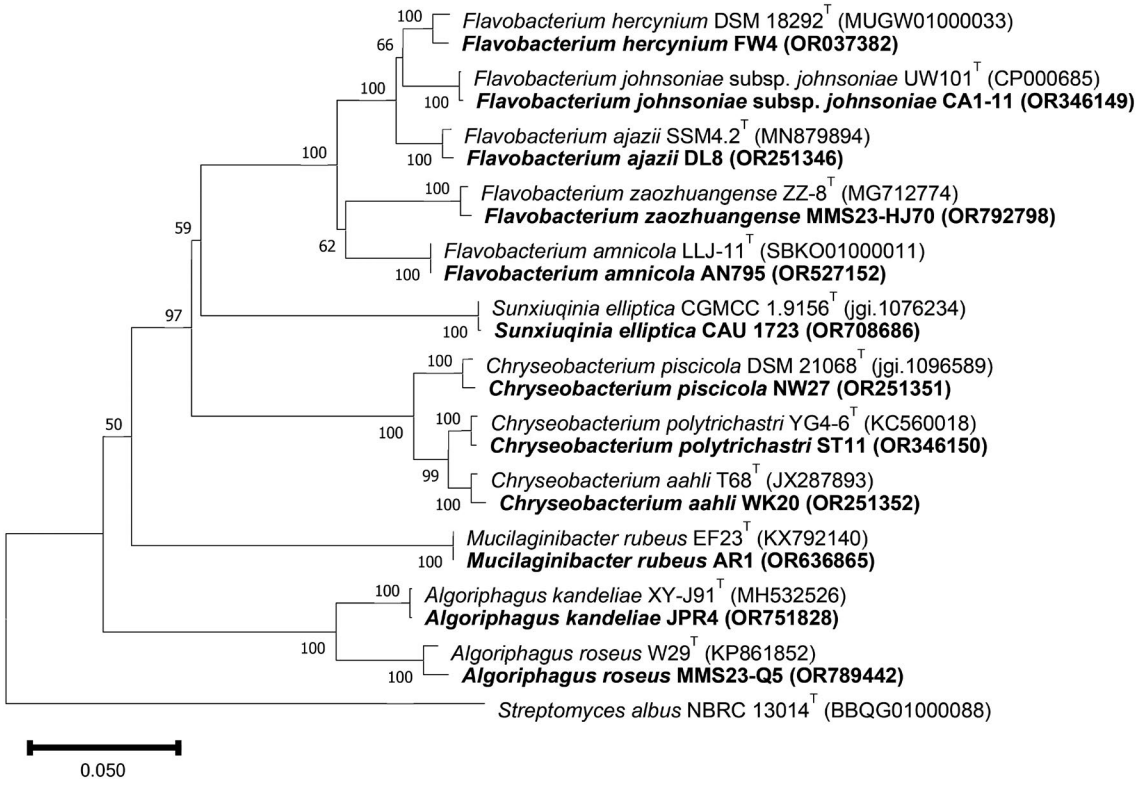


Fig. 3. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the families *Cyclobacteriaceae*, *Flavobacteriaceae*, *Prolixibacteraceae*, *Sphingobacteriaceae* and *Weeksellaceae* of the phylum *Bacteroidota*. Bootstrap values (>50%) are shown at branching points. *Streptomyces albus* NBRC 13014^T used as an outgroup. Scale bar, 0.05 substitutions per nucleotide.

trisodium citrate are utilized as sole carbon sources, but not L-arabinose, capric acid, adipic acid and phenylacetic acid. Strain FW4 (=NIBRBAC000510789) was isolated from an agricultural soil sampled at 994-1 Geumsan-ri, Seomyeon, Chuncheon-si, Gangwon-do, Republic of Korea (37°89′77″N 127°69′35″E). The GenBank accession number for the 16S rRNA gene sequence of strain FW4 is OR037382.

Description of *Flavobacterium zaozhuangense* MMS23-HJ70

Cells are Gram-stain-negative and rod-shaped. Colonies are circular, smooth, glistening and yellow-colored after incubation for 3 days on R2A at 30°C. Positive for β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis and gelatin hydrolysis. D-Glucose is utilized as sole carbon sources, but not 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 MMS23-HJ70 (=NIBRBAC000510812) was isolated from a soil sampled at Gyesan-dong, Gyeyang-gu, Incheon, Republic of Korea (37°54′49″N 126°72′96″E). The GenBank accession number for the 16S rRNA gene sequence of strain MMS23-HJ70 is OR792798.

Description of *Sunxiuqinia elliptica* CAU1723

Cells are Gram-stain-negative and short rod-shaped. Colonies are circular, smooth, entire, convex, opaque and coral-colored after incubation for 3 days on Marine agar at 30°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and gelatin hydrolysis. 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 are not utilized as sole carbon sources. Strain CAU 1723 (=NIBRBAC000510780) was isolated from a mud sampled at 1531, Daebuhwanggeum-ro, Danwon-gu, Ansan-si, Gyeonggi-do, Republic of Korea (37°17′19.8″N 126°34′57.1″E). The GenBank accession number for the 16S rRNA gene sequence of strain CAU 1723 is OR708686.

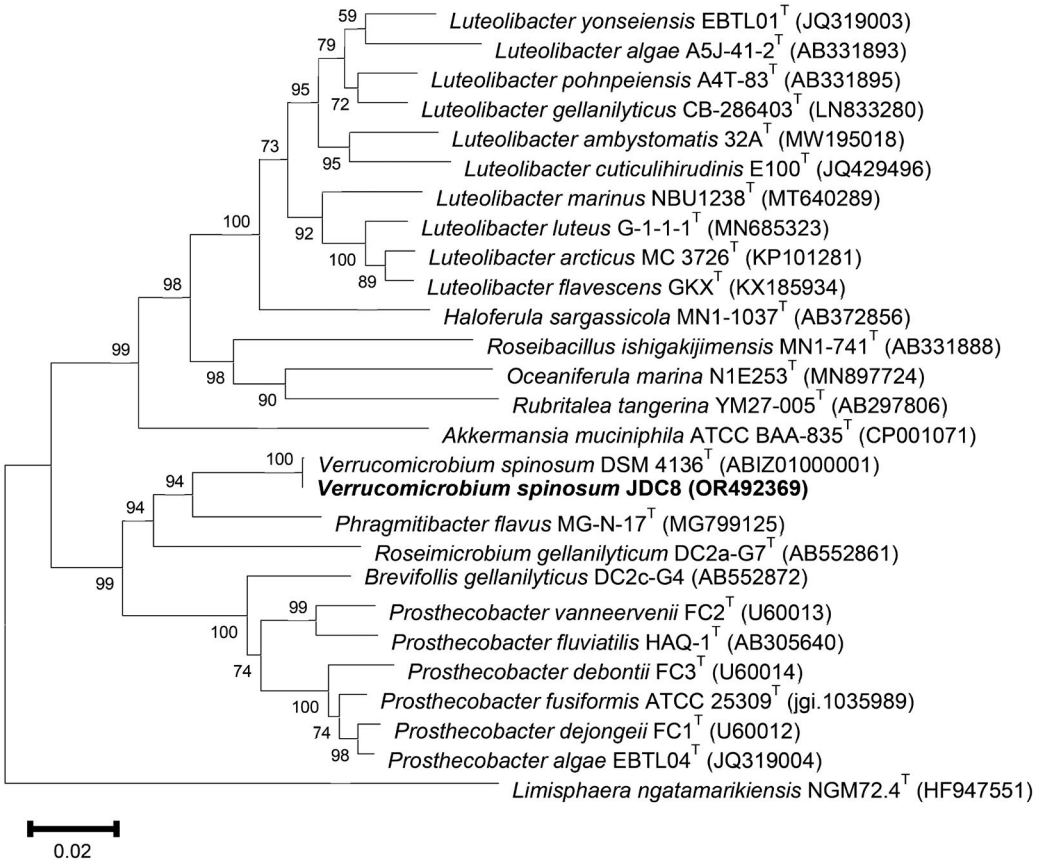


Fig. 4. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study and their relatives in the family *Verrucomicrobiaceae* of the phylum *Verrucomicrobiota*. Bootstrap values (>50%) are shown at branching points. *Limisphaera ngatamarikiensis* NGM72.4^T used as an outgroup. Scale bar, 0.02 substitutions per nucleotide.

Description of *Mucilaginibacter rubeus* AR1

Cells are Gram-stain-negative and short rod-shaped. Colonies are circular, entire, convex, smooth and pink-colored after incubation for 3 days on R2A at 28°C. Positive for nitrate reduction, esculin hydrolysis, gelatin hydrolysis and β-galactosidase, but negative for indole production, glucose fermentation, arginine dihydrolase and urease. D-Glucose and L-arabinose are utilized as sole carbon sources, but not D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Strain AR1 (=NIBRBAC000510794) was isolated from an agricultural soil sampled at Sunhwan-ro, Danwon-gu, Ansan-si, Gyeonggi-do, Republic of Korea (37°34′14″N 126°84′36″E). The GenBank accession number for the 16S rRNA gene sequence of strain AR1 is OR636865.

Description of *Chryseobacterium polytrichastri* ST11

Cells are Gram-stain-negative and rod-shaped. Colonies are circular, slightly convex, glistening and yellow-colored after incubation for 3 days on R2A at 25°C. Positive for esculin hydrolysis and gelatin hydrolysis, and weakly positive for 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, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid are not utilized as sole carbon sources. Strain ST11 (=NIBRBAC000510744) was isolated from a soil sampled at Nowon-gu, Seoul, Republic of Korea (37°38′20.4″N 127°02′53.3″E). The GenBank accession number for the 16S rRNA gene sequence of strain ST11 is OR346150.

Description of *Chryseobacterium aahli* WK20

Cells are Gram-stain-negative and short rod-shaped. Colonies are circular, low convex and golden yellow-colored after incubation for 2 days on R2A at 30°C. Positive for esculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase. D-Glucose is utilized as sole carbon source, but not 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 WK20 (=NIBRBAC000510653) was isolated from a soil sampled at Siksa-dong, Ilsandong-gu, Goyang-si, Gyeonggi-do, Republic of Korea (37°40′47.1″N 126°48′20.3″E). The GenBank accession number for the 16S rRNA gene sequence of strain WK20 is OR251352.

Description of *Chryseobacterium piscicola* NW27

Cells are Gram-stain-negative and rod-shaped. Colonies are round and yellow colored after incubation for 3 days on R2A at 30°C. Positive for esculin hydrolysis and gelatin hydrolysis, but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease and β-galactosidase. 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 are not utilized as sole carbon sources. Strain NW27 (=NIBRBAC000510634) was isolated from a stream sampled at Gongneungcheon, Neungan-ro, Jori-eup, Paju-si, Gyeonggi-do, Republic of Korea (37° 44′29.6″N 126°47′35.2″E). The GenBank accession number for the 16S rRNA gene sequence of strain NW27 is OR251351.

Description of *Verrucomicrobium spinosum* JDC8

Cells are Gram-stain-negative and having wart-like prosthecae in the cell surface. Colonies are circular and yellow-colored after incubation for 2 days on R2A at 30°C. Positive for nitrate reduction, esculin hydrolysis and cytochrome oxidase, but negative for indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, β-galactosidase and cytochrome oxidase. D-Glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, malic acid and trisodium citrate are utilized as sole carbon sources, but not L-arabinose, potassium gluconate, capric acid, adipic acid and phenylacetic acid. Strain JDC8 (=NIBRBAC000510582) was isolated from a soil sampled at Jeongwangcheon-ro, Siheung-si, Gyeonggi-do, Republic of Korea (37°19′54″N 126°44′25″E). The Gen Bank accession number for the 16S rRNA gene sequence of strain JDC8 is OR492369.

Conflicts of Interest

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

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