A report of nine unrecorded bacterial species in the phylum Bacteroidetes collected from freshwater environments in Korea

Sanghwa Park, Kiwoon Beak, Ji-Hye Han, Yoon-Jong Nam and Mi-Hwa Lee*

Bacterial Resources Research Division, Freshwater Bioresources Research Bureau, Nakdonggang National Institute of Biological Resources (NNIBR), Sangju, Gyengsangbuk-do 37242, Republic of Korea

*Correspondent: blume96@nnibr.re.kr

During a comprehensive study of indigenous prokaryotic species in South Korea, nine bacterial species in the phylum Bacteroidetes were isolated from freshwater environmental samples that were collected from three major rivers in the Republic of Korea. High 16S rRNA gene sequence similarity (≥98.7%) and robust phylogenetic clades with the closely related species suggest that each strain was correctly assigned to an independent and predefined bacterial species. There were no previous reports of these nine species in Korea. Within the phylum Bacteroidetes, four species were assigned to the genus Flavobacterium, order Flavobacteriales, and five species to three genera of two families in the order Cytophagales. Gram reaction, colony and cell morphology, basic biochemical characteristics, isolation source, and strain IDs are described in the species description section.

Keywords: 16S rRNA gene, Bacteroidetes, Flavobacteriales, Cytophagales, unrecorded species

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and confirmed using GenBank database (http://ncbi.nlm.nih.gov). For phylogenetic analyses, the 16S rRNA gene sequences were aligned using EzEditor (Jeon et al., 2014). A phylogenetic tree was constructed using the neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-parsimony (MP) (Fitch, 1971) and maximum-likelihood (ML) (Felsenstein, 1981) methods in MEGA version 6 (Tamura et al., 2013). The topology of the phylogenetic tree was evaluated via a bootstrap analysis (Felsenstein, 1985), based on 1,000 replications.

**RESULTS AND DISCUSSION**

On the basis of 16S rRNA gene sequence comparisons and phylogenetic analyses, all nine strains were assigned to the phylum *Bacteroidetes*. The nine strains were distributed in two orders: four strains in the order *Flavobacteriales* and five strains in the order *Cytophagales* (Table 1). These strains were Gram-staining-negative, chemoheterotrophic and rod-shaped bacteria except for strain SJ-153 and BK-168 showing a coccoid or oval shape (Fig. 1). Cultural, morphological and physiological characteristics are also shown in the species description section.

Based on 16S rRNA gene sequence similarity, nine strains were identified as unrecorded bacterial species in Korea. A phylogenetic tree of bacterial strains assigned to the order *Flavobacteriales* and *Cytophagales* in the phylum *Bacteroidetes* are shown in Figs. 2 and 3 respectively. The four species that were assigned to the order *Flavobacteriales* belonged to the genus *Flavobacterium* in the family *Flavobacteriaceae* (Fig. 2). Another five species were assigned to the families *Cyclobacteriaceae* and *Cytophagaceae* in the order *Cytophagales*. Among these species, two species that were assigned to the family *Cyclobacteriaceae* which belongs to the genus *Algoriphagus*. Three species that were assigned to the family *Cytophagaceae* belonged to the genera *Flectobacillus* (1 species) and *Hymenobacter* (2 species) (Fig. 3).

There are no previous reports of these nine species in Korea. In this study, we propose that these species are unrecorded bacterial species: *Flavobacterium ahnfeltiae*, *F. indicum*, *F. macrobrachii*, *F. reichenbachii*, *Algoriphagus alkalophilus*, *A. zhangzhouensis*, *Flectobacillus roseus*, *Hymenobacter algoricola*, and *H. chitinivorans* in Korea.

**Description of Flavobacterium ahnfeltiae SJ-153**

Cells are Gram-staining-negative, non-flagellated and coccus-shaped. Colonies are circular, convex with entire edge and yellow-colored after 3 days on MA at 25°C. Positive for esculin hydrolysis and indole production, but negative for nitrate reduction, glucose fermentation, argi-
nine dihydrolase, urease, gelatinase and β-galactosidase. Does not utilize 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. The strain SJ-513 (=NNIBRBA 38) was isolated from brackish water of Seomjin River, Gwangyang, Korea.

Description of Flavobacterium indicum SS1-37

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex to umbonate with irregular margin and yellow-colored after 3 days on R2A agar at 30°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase, but negative for nitrate reduction, indole production, urease, arginine dihydrolase and glucose fermentation. D-Mannose is utilized. Does not utilize D-glucose, L-arabinose, D-mannitol, malic acid, adipic acid, D-maltose, N-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. The strain SS1-37 (=NNIBRBA 8) was isolated from a riverside sediment of Nakdong River, Gyeong-cheon-island, Sangju, Korea.

Description of Flavobacterium macrobrachii BK-550

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, opaque with entire edge and yellow-colored after 3 days on R2A agar at 25°C. Positive for nitrate reduction, esculin hydrolysis, urease and β-galactosidase, but negative for indole production, gelatinase, glucose fermentation, arginine dihydrolase. D-Glucose, D-mannitol and malic acid are utilized. Does not utilize L-arabinose, D-mannose, D-maltose, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, trisodium citrate and phenylacetic acid.
The strain BK-550 (= NNIBRBA 47) was isolated from freshwater, Eulsuk-island at the end of Nakdong River, Busan, Korea.

**Description of Flavobacterium reichenbachii KS1-10**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, smooth and light yellow-colored after 3 days on R2A agar at 25°C. Positive for urease and arginine dihydrolase, but negative for nitrate reduction, esculin hydrolysis, β-galactosidase, indole production, gelatinase and glucose fermentation. D-Glucose, L-arabinose, D-mannitol, malic acid and adipic acid are utilized. Does not utilize D-mannose, D-maltose, N-acetyl-glucosamine, potassium gluconate, capric acid, trisodium citrate and phenylacetic acid. The strain KS1-10 (= NNIBRBA 55) was isolated from freshwater sediment of Geomyeongso (origin of Han River), Taebaek, Korea.

**Description of Algoriphagus alkaliphilus SJ-361**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex with entire edge and orange-colored after 3 days on MA at 25°C. Positive for nitrate reduction, esculin hydrolysis, gelatinase and β-galactosidase, but negative for urease, glucose fermentation and arginine dihydrolase. D-Glucose, D-mannose, N-acetyl-glucosamine and D-maltose are utilized. Does not utilize L-arabinose, D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain ES1-03 (= NNIBRBA 61) was isolated from brackish marsh sediment of Eulsuk-island at the end of Nakdong River, Busan, Korea.

**Description of Flectobacillus roseus BK-168**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex, entire margins and pale pink-colored after 3 days on R2A agar at 30°C. Positive for esculin hydrolysis and β-galactosidase, but negative for nitrate reduction, indole production, urease,
arginine dihydrolase, gelatinase and glucose fermentation. D-Glucose, D-mannose, N-acetyl-glucosamine, D-maltose and potassium gluconate are utilized. Does not utilize L-arabinose, D-mannitol, malic acid, adipic acid, capric acid, trisodium citrate and phenylacetic acid. The strain BK-168 (= NNIBRBA 26) was isolated from freshwater of Nakdong River, Sangju, Korea.

**Description of Hymenobacter algoricola 04KS1-21**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, smooth and pink-colored after 3 days on R2A agar at 25°C. Positive for esculin hydrolysis, gelatinase and β-galactosidase, but negative for indole production, nitrate reduction, glucose fermentation, arginine dihydrolase and urease. D-Glucose and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain 04KS1-21 (= NNIBRBA 58) was isolated from freshwater sediment of Geomryeongso (origin of Han River), Taebaek, Korea.

**Description of Hymenobacter chitinivorans SJ-175**

Cells are Gram-staining-negative, non-flagellated and rod-shaped. Colonies are circular, convex with entire edge and orange-colored after 3 days on R2A agar at 25°C. Positive for esculin hydrolysis, urease and gelatinase, but negative for indole production, nitrate reduction, glucose fermentation, arginine dihydrolase and β-galactosidase. D-Glucose and D-maltose are utilized. Does not utilize L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid. The strain SJ-175 (= NNIBRBA 39) was isolated from brackish water of Seomjin River, Gwangyang, Korea.

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**References**


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