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A report of 22 unrecorded bacterial species in Korea, isolated from Namhangang

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As part of a larger study of indigenous prokaryotic species diversity in South Korea, various samples from Namhangang were subjected to analyses. Fresh water, underwater sediment, and moss-inhabiting aerobic and anaerobic bacteria were isolated. 22 of the isolates were identified as unrecorded bacterial species in Korea that had ≥98.7% 16S rRNA gene sequence similarity with published species. The aerobic strains isolated were *Kurthia gibsonii* and *Massilia plicata*. Also identified were four facultative anaerobic strains: *Bacillus hisashii*, *Enterococcus rotai*, *Paenibacillus vini*, and *Pediococcus pentosaceus*. 16 strictly anaerobic strains were identified as *Bacteroides xylanolyticus*, *Carnobacterium maltaromaticum*, *Clostridium argentinense*, *Clostridium beijerinckii*, *Clostridium butyricum*, *Clostridium cavendishii*, *Clostridium diolis*, *Clostridium frigidicarnis*, *Clostridium perfringens*, *Clostridium saccharoperbutylacetonicum*, *Clostridium sphenoides*, *Clostridium subterminale*, *Cutibacterium acnes*, *Paraclostridium bifermentans*, *Prevotella paludivivens*, and *Romboutsia lituseburensis*. Based on the examination of morphological, cultural, physiological, and biochemical properties of the isolates, descriptive information of these previously unrecorded species is provided here.

Keywords: anaerobes, Namhangang, unrecorded species

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Introduction

While molecular methods have supplanted traditional microbiological culture-based tools as the favored method of identification of microorganisms, isolation of cultivable bacterial strains is still useful in helping to understand the physiological and functional properties of bacteria (Stewart, 2012). Toward this end, the Korean government is directing projects to examine and gather unrecorded bacterial species in Korea as a part of a larger catalogue of indigenous genetic resources. Through this effort, a number of unrecorded bacterial species have been discovered and registered in the national resource database of Korea (NIBR, 2017).

In this study, we aimed to investigate the diversity of cultivable anaerobic bacteria from freshwater samples, which have been relatively under-studied in previous projects. Six locations in the Namhangang (Namhang River) tributary area were selected as sampling targets: Sinnaecheon, Hanpocheon, Jodaeneub Marshy Land, Yodocheon, Sainam Valley, and Jungnyeongcheon. The four rivers, namely Sinnaecheon, Hanpocheon, Yodocheon, and Jungnyeongcheon, are freshwater rivers with 10.19 km^2, 69.11 km^2, 150.5 km^2, and 130.19 km^2 areas, respectively. The Jodaeneub Marshy Land is downstream of Sinnaecheon and is a wetland that developed as a result of the accumulation of sandy loam along shoals. Sainam Valley is a low land area with streams between cliffs. The six sampling sites are evenly distributed across the Namhangang.

Here, we report 22 newly isolated bacterial strains belonging to the phylum *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria*, all of which are new records for South Korea.

Materials and Methods

Fresh water, underwater sediment, and moss were collected between May 16 and Jun 27, 2017. Sampling sites were Sinnaecheon (N37°27′42.21″; E127°33′16.71″), Hanpocheon (N37°4′44.13″; E127°50′14.83″), Jodaeneub Marshy Land (N37°5′56.85″; E127°49′15.91″), Yodocheon (N36°59′33.79″; E127°45′32.67″), Sainam Valley

(N36°53′32.65″; E128°20′29.58″), and Jungnyeongcheon (N36°11′34.30″; E128°21′4.49″).

To isolate aerobic bacteria, the samples were inoculated and incubated on Reasoner's 2A (R2A; BD) agar medium at 25°C for 1-7 days. To isolate anaerobic bacteria, Anaerobe Basal Agar (ABA; Oxoid) was used and incubated at 30°C for 1-7 days under anaerobic gas condition (80% nitrogen, 10% carbon dioxide, and 10% hydrogen) using anaerobic workstation (Whitley A35, Don Whitley Scientific). Pure cultures of bacterial isolates were maintained using R2A or ABA. The optimum growth temperature was determined by incubating the isolates on corresponding agar medium for up to 1 week. For long-term preservation, the isolates were stored using 20% glycerol suspension at −80°C and lyophilized ampoules. The designation of strains, source of isolation, culture media, and incubation conditions are summarized in Table 1.

PCR amplification and gene sequencing of 16S rRNA gene was done using universal primers (27F, 5′-AGAGTTTGATCMTGGCTCAG-3′ and 1492R, 5′-TACGGYTACCTTGTTACGACTT-3′) as described previously (Shin *et al*., 2016). The sequences were identified by comparing them to the type strain sequence database hosted by the EzBioCloud server (Yoon *et al*., 2017). Based on pairwise sequence similarity, an isolate showing 98.7% or higher similarity to a type strain with a published name, but whose presence has not been reported in Korea, was identified as an unrecorded species.

For phylogenetic analyses, the 16S rRNA gene sequences were aligned using EzEditor program (Jeon *et al*., 2014). Phylogenetic trees were inferred using the neighbor-joining (NJ) and maximum-likelihood (ML) algorithms implemented in MEGA v. 7.0 program (Kumar *et al*., 2016). The Jukes-Cantor model and general time reversible model were used for calculating evolutionary distances of NJ and ML trees, respectively. Bootstrap analysis with 1,000 re-samplings was used to evaluate the trees.

Colonial morphology was observed using cells of 2- to 3-days old on R2A or ABA agar plates. Cellular morphology and cell size were examined by transmission electron microscopy (JEM-3010, Jeol). Gram staining was performed using a Gram-Staining kit (Sigma-Aldrich). Physiological properties were examined by using API 20NE for aerobic strains or API 20A galleries for anaerobic strains (bioMérieux). Oxygen requirement for growth was determined by incubating the inoculated R2A or ABA agar plates at both aerobic and anaerobic conditions.

Results and Discussion

22 isolates, which had at least 98.7% sequence similarity with previously recognized bacterial type strains, were identified as unrecorded bacterial species in the Republic of Korea. The similarity-based identification was further supported by phylogenetic trees (Fig. 1). Each isolate formed a well-supported monophyletic clade with the type strain of identified bacterial species, confirming the proper assignment of the isolate to the species with published names. The tree topology of the maximum-likelihood method was consistent with that of the neighbor-joining tree. The strain information and identification results are summarized in Table 1.

The unrecorded species belonged to the class *Actinobacteria* (1 strain) of the phylum *Actinobacteria*, the class *Betaproteobacteria* (1 strain) of the phylum *Proteobacteria*, the class *Bacteroidia* (2 strains) of the phylum *Bacteroidetes*, the classes *Bacilli* (6 strains), and *Clostridia* (12 strains) of the phylum *Firmicutes*. At generic and family level, those strains belonged to 13 different genera in 11 families: *Bacillus* of *Bacillaceae*, *Carnobacterium* of *Carnobacteriaceae*, *Clostridium* and *Bacteroides* of *Clostridiaceae* and *Lachnospiraceae*, *Enterococcus* of *Enterococcaceae*, *Pediococcus* of *Lactobacillaceae*, *Massilia* of *Oxalobacteraceae*, *Paenibacillus* of *Paenibacillaceae*, *Paraclostridium* and *Romboutsia* of *Peptostreptococcaceae*, *Kurthia* of *Planococcaceae*, *Prevotella* of *Prevotellaceae*, and *Cutibacterium* of *Propionibacteriaceae*.

The cells of isolates were Gram-reaction-negative or positive, rods or cocci, flagellated or non-flagellated bacteria. Colonial colors were white or yellow. None of the isolates produced diffusible pigment on R2A or ABA. Two of the isolates were strict aerobes, four were facultative anaerobes, and 16 were strict anaerobes. Aerobic strains were isolated from moss, and anaerobic strains were isolated from fresh water, underwater sediment, and moss. All the isolates exhibited specific physiological characteristics and enzymatic properties. The detailed feature of carbon source utilization, glucose fermentation, degradation of high molecular weight compounds, and presence of metabolic enzymes are given in the strain descriptions below.

From the results of sequence similarities and phylogenetic trees, we identified 22 strains from the Namhangang samples, including *Bacillus hisashii*, *Bacteroides xylanolyticus*, *Carnobacterium maltaromaticum*, *Clostridium argentinense*, *Clostridium beijerinckii*, *Clostridium butyricum*, *Clostridium cavendishii*, *Clostridium diolis*, *Clostridium frigidicarnis*, *Clostridium perfringens*, *Clostridium saccharoperbutylacetonicum*, *Clostridium sphenoides*, *Clostridium subterminale*, *Cutibacterium acnes*, *Enterococcus rotai*, *Kurthia gibsonii*, *Massilia plicata*, *Paenibacillus vini*, *Paraclostridium bifermentans*, *Pediococcus pentosaceus*, *Prevotella paludivivens*, and *Romboutsia lituseburensis*. These 22 bacterial species have been previously reported in other locations, but are new reports for Korea.

Table 1. Taxonomic affiliations and summary of unrecorded species isolated from Namhangang.

Order	Family	Genus	Strain ID	NNIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation condition
Bacteroidales	*Prevotellaceae*	*Prevotella*	HYN0075	KBA_34	*Prevotella paludivivens*	99.79	Sediment	ABA	30°C, 3d
Burkholderiales	*Oxalobacteraceae*	*Massilia*	HYN0061	KBA_21	*Massilia plicata*	99.64	Moss	R2A	25°C, 2d
Bacillales	*Bacillaceae*	*Bacillus*	HYN0081	KBA_38	*Bacillus hisashii*	99.93	Sediment	ABA	30°C, 2d
	Paenibacillaceae	*Paenibacillus*	HYN0088	KBA_45	*Paenibacillus vini*	99.13	Sediment	ABA	30°C, 3d
	Planococcaceae	*Kurthia*	HYN0065	KBA_25	*Kurthia gibsonii*	99.72	Moss	R2A	25°C, 2d
Lactobacillales	*Carnobacteriaceae*	*Carnobacterium*	HYN0064	KBA_24	*Carnobacterium maltaromaticum*	99.58	Sediment	ABA	30°C, 2d
	Enterococcaceae	*Enterococcus*	HYN0074	KBA_33	*Enterococcus rotai*	100	Fresh water	ABA	30°C, 3d
	Lactobacillaceae	*Pediococcus*	HYN0082	KBA_39	*Pediococcus pentosaceus*	100	Moss	ABA	30°C, 2d
Clostridiales	*Clostridiaceae*	*Clostridium*	HYN0087	KBA_44	*Clostridium argentinense*	98.96	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0084	KBA_41	*Clostridium beijerinckii*	99.93	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0083	KBA_40	*Clostridium butyricum*	99.85	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0062	KBA_22	*Clostridium cavendishii*	99.24	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0068	KBA_28	*Clostridium diolis*	99.78	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0076	KBA_35	*Clostridium frigidicarnis*	99.49	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0080	KBA_37	*Clostridium perfringens*	99.85	Sediment	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0066	KBA_26	*Clostridium saccharoperbutylacetonicum*	99.64	Moss	ABA	30°C, 2d
	Clostridiaceae	*Clostridium*	HYN0077	KBA_36	*Clostridium subterminale*	99.84	Fresh water	ABA	30°C, 2d
	Lachnospiraceae	*Clostridium*	HYN0070	KBA_30	*Clostridium sphenoides*	99.08	Sediment	ABA	30°C, 2d
	Lachnospiraceae	*Bacteroides*	HYN0067	KBA_27	*Bacteroides xylanolyticus*	99.29	Sediment	ABA	30°C, 3d
	Peptostreptococcaceae	*Paraclostridium*	HYN0063	KBA_23	*Paraclostridium bifermentans*	99.41	Sediment	ABA	30°C, 2d
	Peptostreptococcaceae	*Romboutsia*	HYN0072	KBA_31	*Romboutsia lituseburensis*	99.85	Sediment	ABA	30°C, 2d
Propionibacteriales	*Propionibacteriaceae*	*Cutibacterium*	HYN0073	KBA_32	*Cutibacterium acnes*	99.86	Sediment	ABA	30°C, 3d

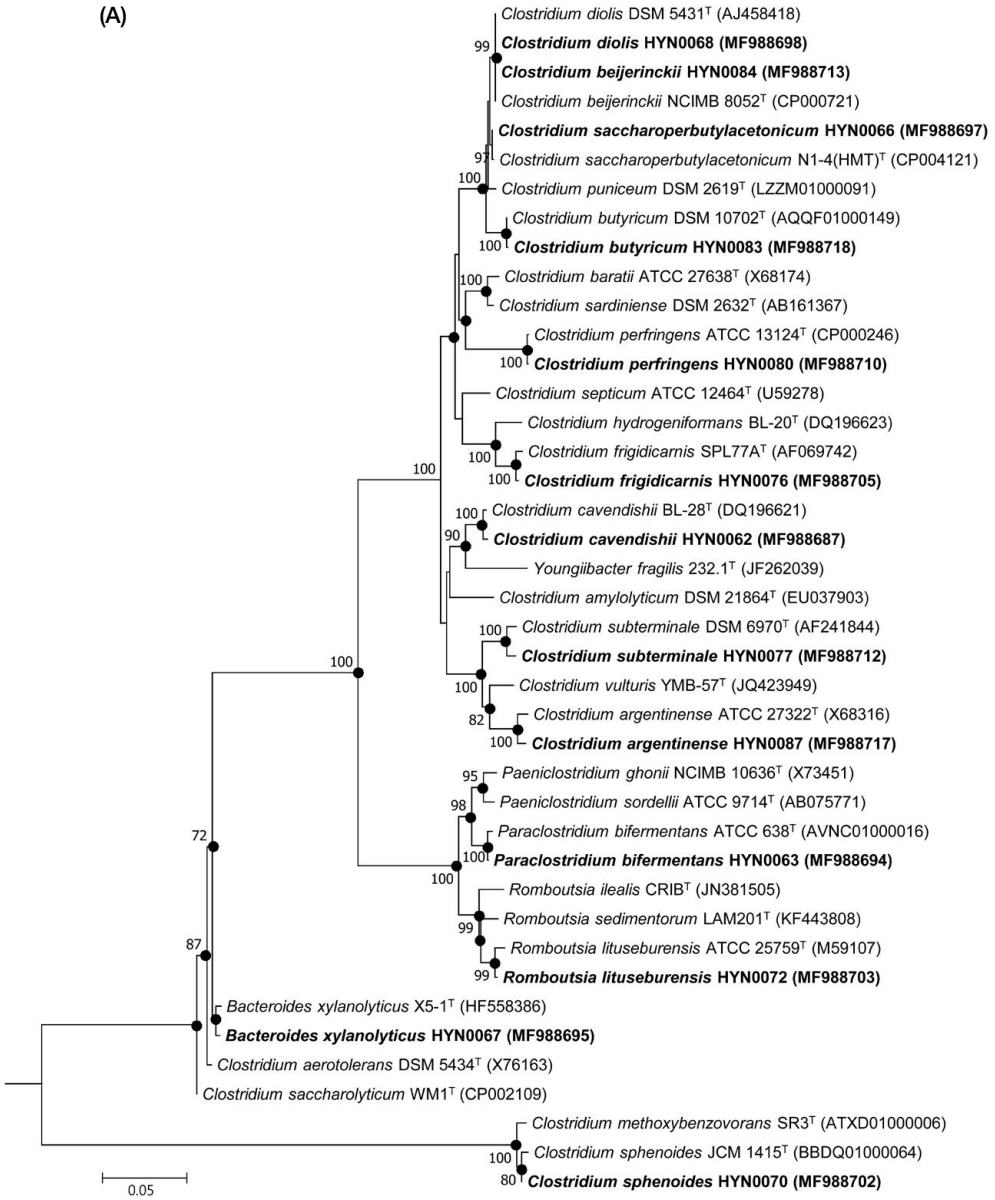

Fig. 1. Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships among the twenty-two isolates and their closest relatives. (A) Isolates belonging to the order *Clostridiales*; (B) isolates belong to the order *Bacteroidales*, *Burkholderiales*, *Bacillales*, *Lactobacillales*, and *Propionibacteriales*. *Thermus amyloliquefaciens* and *Acidolobus aceticus* were used as outgroups. Filled circles indicate the nodes that were also recovered in the maximum-likelihood tree. Bootstrap values are shown above nodes.

Description of *Prevotella paludivivens* HYN0075

Cells are Gram-reaction-negative, non-flagellated, short rod-shaped and approximately 0.6×1.2 μm in size. Colonies are mucoid, convex, and pale white colored after 3 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-mannitol (weakly), D-lactose, D-saccharose (weakly), D-maltose, salicin, D-xylose, L-arabinose, glycerol (weakly), D-cellobiose, D-mannose, D-melezitose (weakly), D-raffinose (weakly), D-sorbitol (weakly), L-rhamnose, and D-trehalose (weakly). Strain HYN0075 (KBA_34) was isolated from the underwater sediment of Yodocheon, Korea.

Description of *Massilia plicata* HYN0061

Cells are Gram-reaction-negative, non-flagellated, rod-shaped and approximately 0.4×0.8 μm in size. Colonies are circular, viscous, and white colored after 2 days on

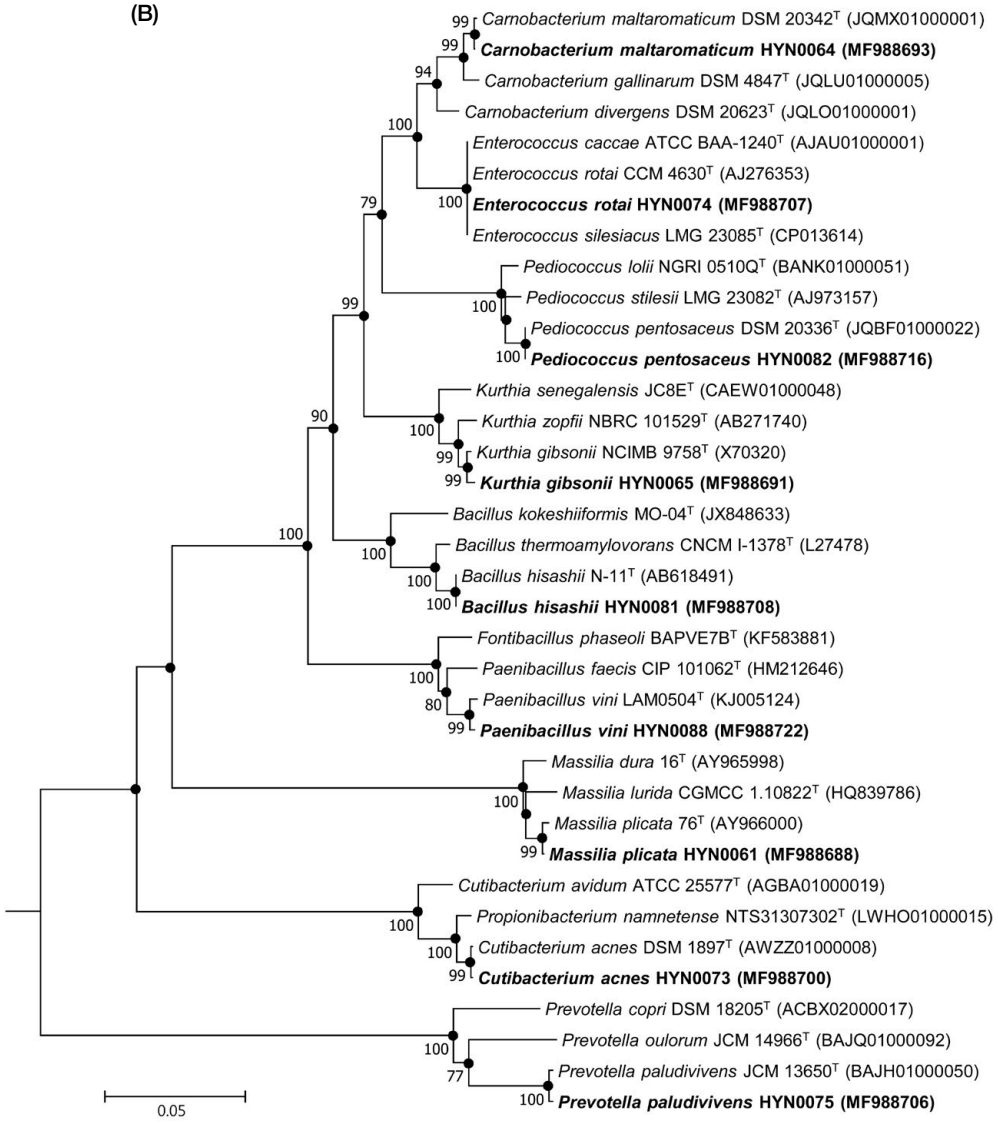

Fig. 1. Continued.

R2A at 25°C. Cells are strict aerobes. Reduces nitrate to nitrite. Possesses activities of oxidase and β-galactosidase, but not urease or arginine dihydrolase. Hydrolyzes esculin and gelatin. Does not produce indole from L-tryptophan. Does not produce acids from glucose. Utilizes D-glucose, L-arabinose, D-mannose, and D-maltose as a sole carbon source, but not from D-mannitol, *N*-acetyl-glucosamine, gluconate, caprate, adipate, malate, citrate, or phenylacetate. Strain HYN0061 (KBA_21) was isolated from a moss of Sinnaecheon, Korea.

Description of *Bacillus hisashii* HYN0081

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.6 × 3.0 μm in size. Colonies are convex and pale yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are facultative anaerobes. Possesses catalase, but not urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0081 (=KBA_38) was isolated from the underwater sediment of Jungnyeongcheon, Korea.

Description of *Paenibacillus vini* HYN0088

Cells are Gram-reaction-negative, non-flagellated,

rod-shaped and approximately 0.6 × 1.2 μm in size. Colonies are circular and pale white colored after 3 days of incubation on anaerobe basal agar at 30°C. Cells are facultative anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin weakly, but not gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0088 (KBA_45) was isolated from the underwater sediment of Yodocheon, Korea.

Description of *Kurthia gibsonii* HYN0065

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.6 × 2.4 μm in size. Colonies are irregular and yellow colored after 2 days on R2A at 25°C. Cells are strict aerobes. Does not reduce nitrate to nitrite. Does not possess activities of oxidase, β-galactosidase, urease, or arginine dihydrolase. Does not hydrolyze esculin or gelatin. Does not produce indole from L-tryptophan. Does not produce acids from glucose. Utilizes *N*-acetyl-glucosamine as a sole carbon source, but not from D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, gluconate, caprate, adipate, malate, citrate, or phenylacetate. Strain HYN0065 (KBA_25) was isolated from a moss of Hanpocheon, Korea.

Description of *Carnobacterium maltaromaticum* HYN0064

Cells are Gram-reaction-positive, non-flagellated, rod-shaped and approximately 0.5 × 1.6 μm in size. Colonies are convex, circular, and pale white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Possesses catalase (weak reaction), but not urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-saccharose, D-maltose, salicin, D-cellobiose, D-mannose, and D-trehalose, weakly from D-mannitol, D-lactose, glycerol, and D-sorbitol, but not from D-xylose, L-arabinose, D-melezitose, D-raffinose, or L-rhamnose. Strain HYN0064 (KBA_24) was isolated from the underwater sediment of Jodaeneub Marshy Land, Korea.

Description of *Enterococcus rotai* HYN0074

Cells are Gram-reaction-positive, non-flagellated, ovoid-shaped and approximately 1.0 × 1.1 μm in size. Colonies are mucoid, convex, and pale white after 3 days of incubation on anaerobe basal agar at 30°C. Cells are facultative anaerobes. Possesses urease, but not catalase. Does not produce indole from L-tryptophan. Hydrolyzes esculin weakly, but not gelatin. Produces acids from D-glucose, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose, but not from D-mannitol. Strain HYN0074 (KBA_33) was isolated from the fresh water of Yodocheon, Korea.

Description of *Pediococcus pentosaceus* HYN0082

Cells are Gram-reaction-positive, non-flagellated, cocci-shaped and approximately 1.1 × 1.5 μm in size. Colonies are circular, convex, and pale white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are facultative anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-lactose, D-maltose, salicin, D-xylose (weakly), L-arabinose, D-cellobiose, D-mannose, L-rhamnose, and D-trehalose, but not from D-mannitol, D-saccharose, glycerol, D-melezitose, D-raffinose, or D-sorbitol. Strain HYN0082 (KBA_39) was isolated from a moss of Hanpocheon, Korea.

Description of *Clostridium argentinense* HYN0087

Cells are Gram-reaction-positive, non-flagellated, rod-shaped and approximately 0.4 × 1.6 μm in size. Colonies are pale yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin and gelatin. Does not produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, or D-trehalose. Strain HYN0087 (KBA_44) was isolated from the underwater sediment of Yodocheon, Korea.

Description of *Clostridium beijerinckii* HYN0084

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.7 × 2.3 μm in size. Colonies are mucoid, circular, and pale yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0084 (KBA_41) was isolated from the underwater sediment of Hanpocheon, Korea.

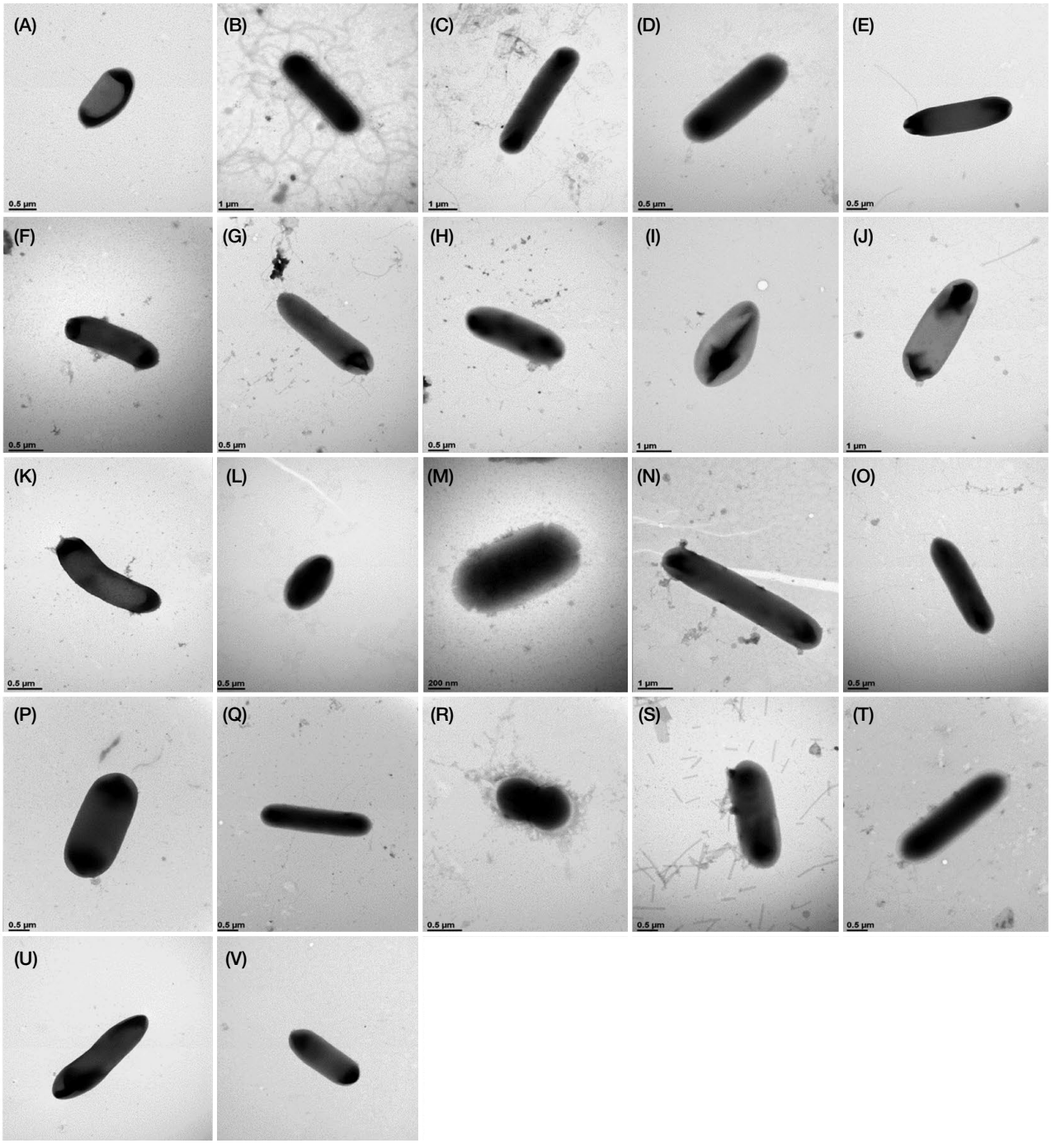

Fig. 2. Transmission electron micrographs of the isolates. Strains: (A) HYN0061; (B) HYN0062; (C) HYN0063; (D) HYN0064; (E) HYN0065; (F) HYN0066; (G) HYN0067; (H) HYN0068; (I) HYN0070; (J) HYN0072; (K) HYN0073; (L) HYN0074; (M) HYN0075; (N) HYN0076; (O) HYN0077; (P) HYN0080; (Q) HYN0081; (R) HYN0082; (S) HYN0083; (T) HYN0084; (U) HYN0087; (V) HYN0088.

Description of *Clostridium butyricum* HYN0083

Cells are Gram-reaction-positive, non-flagellated, rod-shaped and approximately 0.9 × 2.3 μm in size. Colonies are irregular and pale yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xy-

lose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0083 (KBA_40) was isolated from the underwater sediment of Jodaeneub Marshy Land, Korea.

Description of *Clostridium cavendishii* HYN0062

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.2 × 1.5 μm in size. Colonies are convex with lobate margins and creamy white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin and gelatin. Produces acids from D-glucose, D-maltose, salicin, D-cellobiose, D-mannose, and D-trehalose, but not from D-mannitol, D-lactose, D-saccharose, D-xylose, L-arabinose, glycerol, D-melezitose, D-raffinose, D-sorbitol, or L-rhamnose. Strain HYN0062 (KBA_22) was isolated from the underwater sediment of Jodaeneub Marshy Land, Korea.

Description of *Clostridium diolis* HYN0068

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.8 × 2.2 μm in size. Colonies are circular and white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol (weakly), D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0068 (KBA_28) was isolated from the underwater sediment of Hanpocheon, Korea.

Description of *Clostridium frigidicarnis* HYN0076

Cells are Gram-reaction-positive, non-flagellated, rod-shaped and approximately 0.6 × 3.0 μm in size. Colonies are irregular with uneven margins and pale yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Produces acids from D-maltose and glycerol (weakly), but not from D-glucose, D-mannitol, D-lactose, D-saccharose, salicin, D-xylose, L-arabinose, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, or D-trehalose. Strain HYN0076 (KBA_35) was isolated from the underwater sediment of Sainam Valley, Korea.

Description of *Clostridium perfringens* HYN0080

Cells are Gram-reaction-positive, non-flagellated, straight rod-shaped and approximately 1.1 × 2.4 μm in size. Colonies are circular, slightly raised, and yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin and gelatin. Produces acids from D-glucose, D-lactose, D-saccharose, D-maltose, glycerol, D-mannose, D-raffinose (weakly), and D-trehalose, but not from D-mannitol, salicin, D-xylose, L-arabinose, D-cellobiose, D-melezitose, D-sorbitol, or L-rhamnose. Strain HYN0080 (KBA_37) was isolated from the underwater sediment of Jungnyeongcheon, Korea.

Description of *Clostridium saccharoperbutylacetonicum* HYN0066

Cells are Gram-reaction-positive, non-flagellated, straight rod-shaped with rounded ends and approximately 0.8 × 2.9 μm in size. Colonies are circular and white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Possesses catalase, but not urease. Does not produce indole from L-tryptophan. Hydrolyzes gelatin, but not esculin. Produces acids from glycerol, D-mannose, and D-sorbitol, but not from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, D-cellobiose, D-melezitose, D-raffinose, L-rhamnose, or D-trehalose. Strain HYN0066 (KBA_26) was isolated from a moss of Sinnaecheon, Korea.

Description of *Clostridium subterminale* HYN0077

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.6 × 2.3 μm in size. Colonies are irregular and pale white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Does not hydrolyze esculin or gelatin. Produces acids from D-glucose weakly, but not from D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, or D-trehalose. Strain HYN0077 (KBA_36) was isolated from the fresh water of Yodocheon, Korea.

Description of *Clostridium sphenoides* HYN0070

Cells are Gram-reaction-positive, non-flagellated, oval-shaped and approximately 0.9 × 1.6 μm in size. Colonies circular and white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Produce indole from L-tryptophan weakly. Hydrolyzes esculin,

but not gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin (weakly), D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose (weakly), D-raffinose, D-sorbitol (weakly), L-rhamnose, and D-trehalose. Strain HYN0070 (KBA_30) was isolated from the underwater sediment of Jungnyeongcheon, Korea.

Description of *Bacteroides xylanolyticus* HYN0067

Cells are Gram-reaction-negative, flagellated, rod-shaped and approximately 0.6 × 2.5 μm in size. Colonies are mucoid and pale white colored after 3 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin, but not gelatin. Does not produce acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0067 (KBA_27) was isolated from the underwater sediment of Hanpocheon, Korea.

Description of *Paraclostridium bifermentans* HYN0063

Cells are Gram-reaction-positive, flagellated, rod-shaped and approximately 0.4 × 1.6 μm in size. Colonies are irregular, mucoid, and pale yellow colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Produces indole from L-tryptophan. Hydrolyzes esculin and gelatin. Produces acids from D-glucose, D-maltose, D-mannose, and D-sorbitol (weakly), but not from D-mannitol, D-lactose, D-saccharose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-melezitose, D-raffinose, L-rhamnose, or D-trehalose. Strain HYN0063 (KBA_23) was isolated from the underwater sediment of Jodaeneub Marshy Land, Korea.

Description of *Romboutsia lituseburensis* HYN0072

Cells are Gram-reaction-negative, flagellated, short rod-shaped and approximately 0.6 × 1.7 μm in size. Colonies are circular and white colored after 2 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Does not possess catalase or urease. Does not produce indole from L-tryptophan. Hydrolyzes esculin and gelatin. Produces acids from D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose. Strain HYN0072 (KBA_31) was isolated from the underwater sediment of Jungnyeongcheon Rive, Korea.

Description of *Cutibacterium acnes* HYN0073

Cells are Gram-reaction-positive, non-flagellated, rod-shaped and approximately 0.4 × 1.89 μm in size. Colonies are mucoid, convex, and pale yellow colored after 3 days of incubation on anaerobe basal agar at 30°C. Cells are strict anaerobes. Possesses catalase, but not urease. Does not produce indole from L-tryptophan. Hydrolyzes gelatin, but not esculin. Produces acids from D-glucose, glycerol, D-mannose, and D-sorbitol, but not from D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, D-cellobiose, D-melezitose, D-raffinose, L-rhamnose, or D-trehalose. Strain HYN0073 (KBA_32) was isolated from the underwater sediment of Sainam Valley, Korea.

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References

Jeon, Y.S., K. Lee, S.C. Park, B.S. Kim, Y.J. Cho, S.M. 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.

Kumar, S., G. Stecher and K. Tamura. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33:1870-1874.

NIBR 2017. National List of Species of Korea, National Institute of Biological Resources.

Shin, S.K., E. Kim, S. Choi and H. Yi. 2016. Cochleicola gelatinilyticus gen. nov., sp. nov., Isolated from a Marine Gastropod, Reichia luteostoma. J Microbiol Biotechnol 26:1439-1445.

Stewart, E.J. 2012. Growing unculturable bacteria. J Bacteriol 194:4151-4160.

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 gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67: 1613-1617.

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