A report of 12 unrecorded prokaryotic species isolated from gastrointestinal tracts and feces of various endangered animals in Korea

Pil Soo Kim^{1,§}, Ki-Eun Lee^{2,§}, Euon Jung Tak¹, Myung-Suk Kang³ and Jin-Woo Bae^{1,*}

In 2016 and 2017, as part of a comprehensive investigation to identify the prokaryotic species in Korea, a total of 12 bacterial strains were isolated from the gastrointestinal tract and/or fecal samples of four endangered species, including reptile, bird, and marine and terrestrial mammals. Phylogenetic analysis with the 16S rRNA gene sequence was used to assign these strains to the phyla, Firmicutes, Actinobacteria or Proteobacteria. Furthermore, most of the strains Firmicutes belonged to the order *Lactobacillales*. Interestingly, 12 of the isolated strains have not been previously reported from the Korean Peninsula. Also, based on their high 16S rRNA gene sequence similarities (>98.7%) and formation of strong monophyletic clades with the closest type species, each isolated strain of isolates was assigned to an independent, predefined bacterial species. Gramstain reaction, colony and cell morphology, biochemical characteristics, isolation source, and NIBR IDs are described in the species description section.

Keywords: 16S rRNA sequence, endangered animals, gut microbiota, Korean Peninsula, unrecorded species

© 2020 National Institute of Biological Resources DOI:10.12651/JSR.2020.9.1.035

Introduction

At present, it is estimated that there are more than 1.5 million animal species on Earth. However, since the beginning of the Industrial Revolution, the natural habitats of wild animals have come under threat from environmental disruption and climate change (Baillie *et al.*, 2004), and currently approximately 20,000 species of animal are threatened with extinction. In South Korea, the Ministry of Environment has designated 246 animal species as endangered, and considerable effort and resources are being spent on conservation programs.

Symbiotic microbiota helps animals adapt to different habitats and niches by providing metabolic benefits and promoting homeostasis (Bäckhed *et al.*, 2005; Turnbaugh *et al.*, 2006). For example, the gut microbiota aids digestive functioning by promoting intestinal epithelial proliferation, nutrient absorption and energy conversion, and also affords protection against entero-pathogenic bacteria (Bäckhed *et al.*, 2004; Stecher *et al.*, 2007; Hoffmann *et*

al., 2009; Round and Mazmanian, 2009).

In 2016–2017, we investigated the gut microbiota of various endangered animals living on the Korean Peninsula. Hundreds of bacterial species were isolated and among these species, several unreported bacterial species in South Korea were identified. We characterized the isolates with various phenotypic and genotypic identification, and phylogenetic analysis. From the gut microbiota of the endangered animal species that were included in our study, we report 12 bacterial species that have not been recorded from the Korean Peninsula.

MATERIALS AND METHODS

Strain isolation and characterization

Twelve unreported bacterial strains were isolated from the gastrointestinal tract (GIT) and/or feces of various endangered species (green sea turtle, finless porpoise, Siberian musk deer, and Andean condor) found on the Korean

¹Department of Biology and Department of Life and Nanopharmaceutical Sciences, Kyung Hee University, Seoul 02447, Republic of Korea

²Microorganism Resources Division, National Institute of Biological Resources, Incheon 22689, Republic of Korea

³Biological Resources Utilization Department, National Institute of Biological Resources, Incheon 22689, Republic of Korea

^{*}Correspondent: baejw@khu.ac.kr

[§]These authors contributed equally to this work.

Peninsula, which were collected by National Institute of Biological Resources and Seoul Grand Zoo. These isolates were assigned to the phyla Firmicutes, Bacteroidetes, or Proteobacteria, and of the Firmicutes, most belong to the order *Lactobacillales* (Table 1). Samples were collected under aseptic conditions. Homogenized GIT tissue and fecal samples were serially diluted with sterile phosphate buffered saline (PBS) and spread onto different culture media (Reasoner's 2A Agar [R2A], Marine Agar 2216 [MA], Tryptic Soy Agar [TSA], and Brain heart infusion [BHI] agar], supplemented with 5% sheep blood, and incubated for at 25-30°C for 1 week. Isolated, single bacterial colonies were prepared using the streak-plating method. The colony morphology and cell size were recorded after incubation on the appropriate growth media (R2A, TSA or MA) at 25-30°C for 2 days. Transmission electron micrographs (obtained using the LIBRA 120, transmission electron microscope, Carl Zeiss) of the isolated strains are shown in Figure 1. Biochemical analysis and gram-staining were performed using API kits (API 20NE, API ZYM and API ID 32GN; bioMerieux), GEN III microplates (Biolog) and a Gram-stain kit according to manufacturer's instructions. The results are shown in Table 1 and in the strain description (below).

Analysis of 16S ribosomal RNA gene sequence

For selected pure isolate colonies, genomic DNA was extracted using the AccuPrep Genomic DNA Extraction kit (Bioneer, Korea) or UltraClean Microbial DNA Isolation Kit (MoBio, USA). Amplification of the 16S ribosomal RNA (16S rRNA) gene sequences was performed using the PCR Premix (iNtron Biotechnology, Korea) and bacterial, universal forward and reverse primers (forward primer 27F, 5'-AGAGTTTGATCCTGGCTCAG-3'; reverse primer 1088R 5'-GCTCGTTGCGGGACTTA-ACC-3' or 1492R 5'-GGYTACCTTGTTACGACTT-3') (Lane, 1991). The 16S rRNA gene amplicons were sequenced by a certified service provider (Macrogen, Korea) using an automated DNA analyzer (Applied Biosystems 3730xl DNA Analyzer). DNA sequences were assembled using SeqMan (DNASTAR) and near full-length 16S rRNA gene sequences compared with the 16S rRNA gene sequences of bacterial reference strains using the EzBio-Cloud database (Yoon et al., 2017).

Phylogenetic analysis

The 16S rRNA gene sequences of the bacterial isolates were aligned with the corresponding sequences of bacterial reference strains using the BioEdit software with the multiple alignment algorithm [CLUSTAL W; (Thompson et al., 1994; Hall, 1999)]. Phylogenetic trees, using the 16S rRNA gene sequences of the isolates and the closely related bacterial species were constructed using the

MEGA 7 software (Kumar *et al.*, 2016). Neighbor-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods with 1000 bootstrap replicates were used to ascertain phylogenetic correlations (Kluge and Farris, 1969; Felsenstein, 1981; 1985; Saitou and Nei, 1987).

RESULTS AND DISCUSSION

Based on the phylogenetic analysis of the 16S rRNA gene sequences, the 12 isolated strains (AE4-1, B3, M3, M3R204, M3R205, M1T305, M1T307, VT2414, VT2418, VT2504, VM2501, and VM3408) were assigned to the phyla Firmicutes, Bacteroidetes or Proteobacteria. The isolated strains were confirmed as chemoheterotrophic and rod or coccoid-shaped bacteria (Fig. 1). The morphological, physiological, and biochemical characteristics of are isolated strains are described in detail below.

Based on the 16S rRNA gene sequences, the 12 strains isolated in our study (strains AE4-1, B3, M3, M3R204, M3R205, M1T305, M1T307, VT2414, VT2418, VT2504, VM2501, and VM3408) were most closely related to Enterococcus thailandicus DSM 21767^T (JXLE01000039; 100% sequence identity); Vagococcus fessus M2661/98/1^T (AJ243326; 100% sequence identity); Lactobacillus sakei subsp. carnosus DSM 15831^T (AZFG01000015; 100% sequence identity); Microbacterium oxydans DSM 20578^T (Y17227; 99.85% sequence similarity); Ochrobactrum pituitosum CCUG 50899^T (AM490609; 99.62% sequence similarity); Lysinibacillus mangiferihumi M-GX18^T (JF731238; 99.19%), Streptococcus gallolyticus subsp. macedonicus ACA-DC 206^T (Z94012; 99.70% sequence similarity); Rhodococcus phenolicus DSM 44812^T (LRRH01000094; 98.85% sequence similarity); Brevibacterium siliguriense DSM 23676^T (LT629766; 99.35% sequence similarity); Glutamicibacter mysorens LMG 16219^T (AJ639831; 99.35% sequence similarity; Arthrobacter rhombi F.98.3HR.69^T (Y15885; 99.35%); and Escherichia marmotae HT073016^T (JNBP01000188; 99.26% sequence similarity), respectively. A phylogenetic analysis of the isolated bacterial strains was performed based on 16S rRNA gene sequences. In the consensus phylogenetic tree, isolated strains formed robust phylogenetic clades with the most closely related species in the phyla Firmicutes, Bacteroidetes, and Proteobacteria and order Lactobacillales, as expected from high 16S rRNA gene sequence similarities (Fig. 2).

Description of Enterococcus thailandicus AE4-1

Cells are Gram-staining positive, non-flagellated, and coccus. Colonies are circular, raised, entire, and white colored after 2 days of incubation on MRS agar at 30°C. Positive for adipate, malate in API 20NE, but negative

Table 1. Summary of the isolated strains from the gastrointestinal tract of the endangered species in Korea and their taxonomic affiliations.

								Most closely 1	Most closely related species	147	
Phylum	Class	Order	Family	Genus	Strain ID	NIBR ID	Accession	Closest type strain	Accession	Similarity (%)	Isolation source
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	AE4-1	NIBRBAC000503069 MN524154	MN524154	Enterococcus thailandicus	JXLE01000039	100	Green sea turtle (Chelonia mydas)
Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Vagococcus	В3	NIBRBAC000503068 MN524155		Vagococcus fessus	NGJY01000008	100	Finless porpoise (Neophocaena phocaenoides)
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae	Lactobacillus	M3	NIBRBAC000503070 MN524156 Lactobacillus sakei subsp. carnosus	MN524156	Lactobacillus sakei subsp. carnosus	AZFG01000015	100	Finless porpoise (Neophocaena phocaenoides)
Actinobacteria	Actinobacteria Actinobacteria	Micrococcales	Microbacteriaceae		M3R204	Microbacterium M3R204 NIBRBAC000499672	KX881415	Microbacterium oxydans	Y17227	99.85	Siberian musk deer (Moschus moschiferus)
Proteobacteria	Proteobacteria Alphaproteobacteria	Rhizobiales	Brucellaceae	Ochrobactrum	M3R205	NIBRBAC000499689 KX881416		Ochrobactrum pituitosum	AM490609	99.62	Siberian musk deer (Moschus moschiferus)
Firmicutes	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	M1T305	NIBRBAC000499685 KX881417		Lysinibacillus mangiferihumi	JF731238	99.2	Siberian musk deer (Moschus moschiferus)
Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	M1T307	NIBRBAC000499688 KX881418		Streptococcus gallolyticus subsp. macedonicus	UHFM01000006	02.66	Siberian musk deer (Moschus moschiferus)
Actinobacteria	Actinobacteria Actinobacteria	Mycobacteriales Nocardiaceae	Nocardiaceae	Rhodococcus	VT2414	NIBRBAC000499827	MF480439	Rhodococcus phenolicus	LRRH01000094	98.85	Andean condor (Vultur gryphus)
Actinobacteria	Actinobacteria Actinobacteria	Micrococcales	Brevibacteriaceae	Brevibacterium	VT2418	NIBRBAC000499828	MF480440	Brevibacterium siliguriense	LT629766	99.35	Andean condor (Vultur gryphus)
Actinobacteria	Actinobacteria Actinobacteria	Micrococcales	Micrococcaaceae	Glutamicibacter	VT2504	NIBRBAC000499829	MF480441	Glutamicibacter mysorens	AJ639831	99.35	Andean condor (Vultur gryphus)
Actinobacteria	Actinobacteria Actinobacteria	Micrococcales	Micrococcaaceae	Arthrobacter	VM2501	NIBRBAC000499830 MF480442	MF480442	Arthrobacter rhombi	Y15885	99.35	Andean condor (Vultur gryphus)
Proteobacteria	Proteobacteria Gamnaproteobacteria Enterobacteriales Enterobacteriaceae Escherichia	Enterobacteriales	Enterobacteriaceae	Escherichia	VM3408	VM3408 NIBRBAC000499831 MF480443	MF480443	Escherichia marmotae	JNBP01000188	99.26	Andean condor (Vultur gryphus)

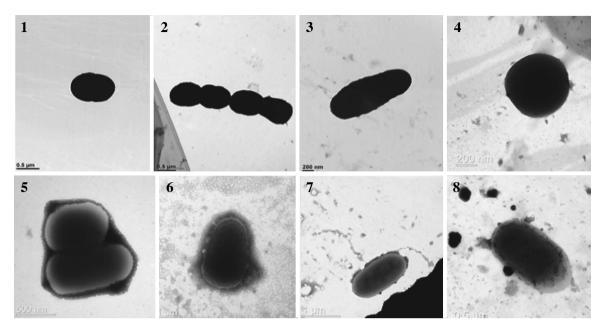


Fig. 1. Transmission electron micrographs of the isolated strains. Strain-1, AE4-1; strain-2, B3; strain-3, M3; strain-4, VM3408; strain-5, VT2418; strain-6, VM2501; strain-7, VT2414; strain-8, VT2504.

for nitrate reduction, reduction of nitrates to nitrogen, indole production, glucose acidification, arginine dihydrolase, urease, β-glucosidase, protease, β-galactosidase, D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconate, caprate, citrate, and phenyl-acetate. D-sorbitol, L-histidine, 4-hydroxy-benzoate, D-sucrose, and acetate are utilized. Does not utilize D-mannitol, D-glucose, salicin, D-melibiose, L-fucose, L-arabinose, propionate, caprate, valerate, citrate, 2-ketogluconate, 3-hydroxy-butyrate, L-proline, L-rhamnose, D-ribose, N-acetyl-glucosamine, inositol, D-maltose, itaconate, suberate, malonate, D, L-lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine.

Strain AE4-1 (= NIBRBAC000503069) was isolated from the feces of a green sea turtle (*Chelonia mydas*), Chungcheongnam Province, Korea.

Description of Vagococcus fessus B3

Cells are Gram-staining positive, non-flagellated, and coccus. Colonies are circular, raised, entire, and white colored after 2 days of incubation on MRS agar at 30°C. Positive for nitrate reduction, β -glucosidase, D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose, gluconate, malate, and citrate in API 20NE, but negative for indole production, glucose acidification, arginine dihydrolase, urease, protease, β -galactosidase, D-mannitol, caprate, adipate, phenyl-acetate. D-glucose, salicin, D-melibiose, L-arabinose, citrate, 2-ketogluconate, 3-hydroxy-butyrate, L-proline, L-rhamnose, N-acetyl-glu-

cosamine, inositol, D-maltose, itaconate, and suberate are utilized. Does not utilize D-mannitol, L-fucose, D-sorbitol, propionate, caprate, valerate, L-histidine, 4-hydroxy-benzoate, D-ribose, D-sucrose, malonate, acetate, D, L-lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine.

Strain B3 (=NIBRBAC000503068) was isolated from the intestinal tract of a finless porpoise (*Neophocaena phocaenoides*), Chungcheongnam Province, Korea.

Description of Lactobacillus sakei subsp. carnosus M3

Cells are Gram-staining positive, non-flagellated, and rod. Colonies are circular, raised, entire, and white colored after 2 days of incubation on MRS agar at 30°C. Positive for β -glucosidase, L-arabinose in API 20NE, but negative for nitrate reduction, reduction of nitrates to nitrogen, indole production, glucose acidification, arginine dihydrolase, urease, protease, β -galactosidase, D-glucose, D-mannose, D-mannitol, N-acetyl-glucosamine, D-maltose, gluconate, caprate, adipate, malate, citrate, phenyl-acetate. D-melibiose and L-arabinose are utilized. Does not utilize D-mannitol, D-glucose, salicin, L-fucose, D-sorbitol, propionate, caprate, valerate, citrate, L-histidine, 2-ketogluconate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, N-acetyl-glucosamine, D-ribose, inositol, D-sucrose, D-maltose, itaconate, suberate, malonate, acetate, D, L-lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine.

Strain M3 (= NIBRBAC000503070) was isolated from the intestinal tract of a finless porpoise (*Neophocaena*

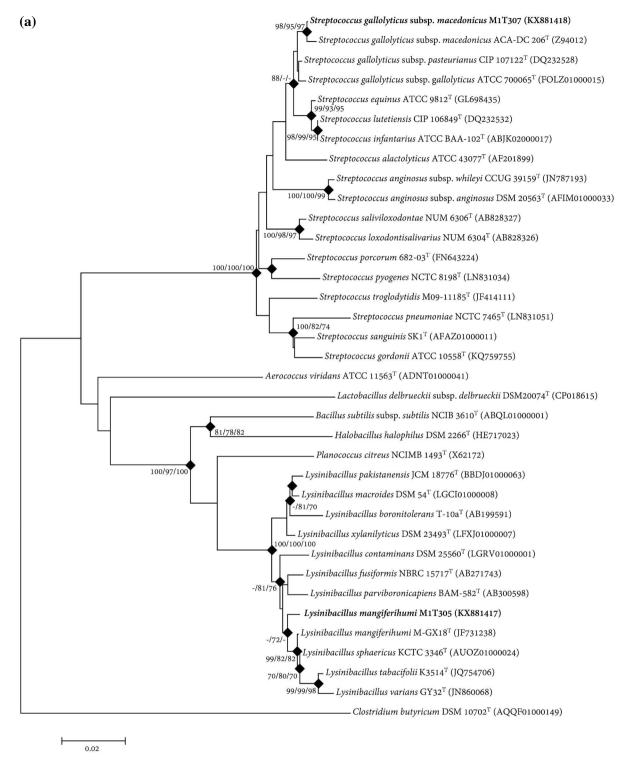


Fig. 2. Phylogenetic tree based on 16S rRNA gene sequence comparisons, showing the relationship between the isolated strains in this study and the notable species from phylum Firmicutes (a) order *Lactobacillales* (In particular *Enterococcus*, *Lactobacillus* and *Vagococcus*), phylum Actinobacteria (c) and phylum Proteobacteria (d) and. The trees were mainly reconstructed using the neighbor-joining algorithm (NJ), Maximum parsimony (MP) and maximum likelihood (ML) algorithms were applied for additional comparison. Filled diamonds indicate branches present in the phylogenetic trees generated using the three different methods. Numbers on the nodes (>70%) represent bootstrap values as percentages of 1000 replicates (NJ/MP/ML). *Clostridium butyricum* DSM 10702^T (AQQF01000149), *Bifidobacterium bifidum* ATCC 29521^T (KE993182) and *Spirochaeta aurantia* subsp. *aurantia* DSM 1902^T (FR749896) were used as outgroups, respectively. Bar, 0.02 (a, c, d) and 0.01 (b) accumulated changes per nucleotide.

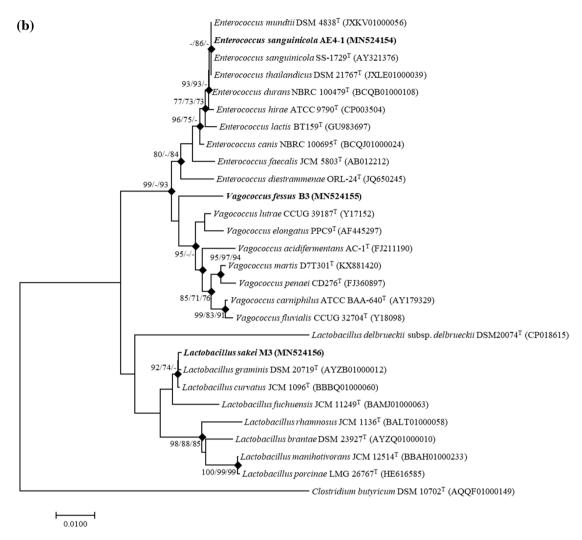


Fig. 2. Continued.

phocaenoides), Chungcheongnam Province, Korea.

Description of Microbacterium oxydans M3R204

Cells are Gram-staining positive. Colonies are circular, raised, entire, and white colored after 2 days of incubation on R2A agar at 20°C. Dextrin, D-Maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-salicin, D-mannose, D-fructose, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, α-D-glucose, D-galactose, L-rhamnose, inosine, 1% sodium lactate, myo-inositol, glycerol, rifamycin SV, L-alanine, L-histidine, guanidinium chloride, pectin, tetrazolium blue, methyl pyruvate, nalidixic acid, lithium chloride, α-keto-butyric acid, acetoacetic acid, acetic acid, sodium butyrate, D-mannitol, glycyl-L-proline, L-aspartic acid, L-glutamic acid, L-lactic acid, D-malic acid, L-malic acid, bromosuccinic acid,

Tween 40, α -hydroxybutyric acid, propionic acid, aztreonam, and sodium bromate are utilized as the sole source of carbon. α -D-lactose, β -methyl-D-glucoside, N-acetyl- β -D-mannosamine, 3-methyl glucose, D-fucose, L-fucose, fusidic acid, D-serine, D-glucose-6-phosphate, D-fructose-6-phosphate, troleandomycin, minocycline, lincomycin, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, glucuronamide, vancomycin, tetrazolium violet, potassium tellurite, stachyose, D-raffinose, D-melibiose, N-acetyl neuraminic acid, D-sorbitol, D-arabitol, D-aspartic acid, D-serine, gelatin, L-arginine, L-pyroglutamic acid, niaproof 4, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, citric acid, α -keto-glutaric acid, γ -aminobutryric acid, β -hydroxy-D, L-butyric acid, and formic acid are not utilized as the sole source of carbon (Biolog GEN III).

Strain M3R204 (=NIBRBAC000499672) was isolated from the feces of a Siberian musk deer (*Moschus*

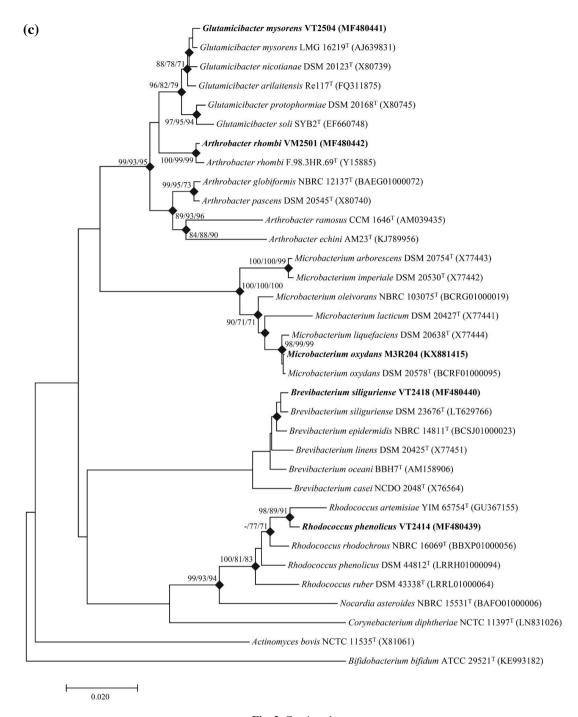


Fig. 2. Continued.

moschiferus), Hwacheon, Gangwon Province, Korea. The DNA G+C content of the type strain is 72.9 mol%.

Description of Ochrobactrum pituitosum M3R205

Cells are Gram-staining positive. Colonies are circular, smooth, convex, and yellowish-white colored after 2 days of incubation on R2A agar at 20°C. Dextrin, *N*-ace-

tyl-D-glucosamine, *N*-acetyl-D-galactosamine, *α*-D-glucose, D-mannose, D-fructose, D-galactose, L-rhamnose, inosine, 1% sodium lactate, *myo*-inositol, rifamycin SV, L-alanine, L-histidine, guanidinium chloride, tetrazolium blue, methyl pyruvate, lithium chloride, acetoacetic acid, acetic acid, glycyl-L-proline, L-aspartic acid, L-glutamic acid, L-serine, L-lactic acid, D-malic acid, L-malic acid, bromosuccinic acid, propionic acid, aztreonam, D-fucose,

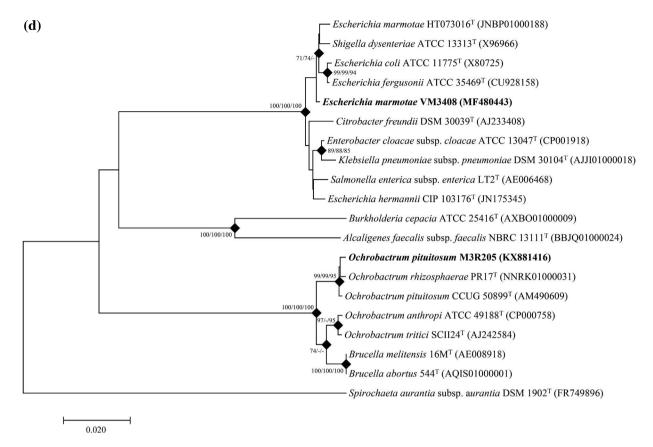


Fig. 2. Continued.

L-fucose, troleandomycin, lincomycin, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, vancomycin, tetrazolium violet, potassium tellurite, D-sorbitol, D-arabitol, niaproof 4, citric acid, and γ -aminobutryric acid are utilized as the sole source of carbon. However, D-maltose, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-salicin, glycerol, pectin, nalidixic acid, α-keto-butyric acid, sodium butyrate, D-mannitol, D-gluconic acid, 4-hydroxyphenylacetic acid, Tween 40, α -hydroxybutyric acid, sodium bromate, α -D-lactose, β -methyl-D-glucoside, N-acetyl- β -D-mannosamine, 3-methyl glucose, fusidic acid, D-serine, D-glucose-6-phosphate, D-fructose-6-phosphate, minocycline, glucuronamide, stachyose, D-raffinose, D-melibiose, N-acetyl neuraminic acid, D-aspartic acid, D-serine, gelatin, L-arginine, L-pyroglutamic acid, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, α -keto-glutaric acid, β -hydroxyl-D, L-butyric acid, and formic acid are not utilized as the sole source of carbon (Biolog GEN III).

Strain M3R205 (=NIBRBAC000499689) was isolated from the feces of a Siberian musk deer (*Moschus moschiferus*), Hwacheon, Gangwon Province, Korea. The DNAG+C content of the type strain is 55.0 mol%.

Description of Lysinibacillus mangiferihumi M1T305

Cells are Gram-staining positive. Colonies are circular, smooth, convex, and yellowish-white colored after 2 days of incubation on R2A agar at 37°C. Dextrin, N-acetyl-D-glucosamine, N-acetyl-D-galactosamine, D-fructose, D-galactose, L-rhamnose, inosine, 1% sodium lactate, myo-inositol, L-alanine, L-histidine, methyl pyruvate, lithium chloride, acetoacetic acid, acetic acid, glycyl-L-proline, L-aspartic acid, L-glutamic acid, L-serine, L-lactic acid, D-malic acid, L-malic acid, bromosuccinic acid, propionic acid, aztreonam, D-fucose, L-fucose, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, potassium tellurite, D-arabitol, citric acid, γ -aminobutryric acid, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-salicin, glycerol, α -keto-butyric acid, sodium butyrate, D-mannitol, D-gluconic acid, Tween 40, α -hydroxybutyric acid, β -methyl-D-glucoside, N-acetyl- β -D-mannosamine, 3-methyl glucose, D-serine, D-glucose-6-phosphate, D-fructose-6-phosphate, glucuronamide, stachyose, D-melibiose, N-acetyl neuraminic acid, D-aspartic acid, D-serine, gelatin, L-arginine, L-pyroglutamic acid, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, α -keto-glutaric acid, β -hydroxy-D, L-butyric acid, and formic acid are utilized as the sole source of carbon. However, α -D-glucose, D-mannose, rifamycin SV, guanidinium chloride, tetrazolium blue, troleandomycin, lincomycin, vancomycin, tetrazolium violet, D-sorbitol, niaproof 4, D-maltose, pectin, nalidixic acid, 4-hydroxyphenylacetic acid, sodium bromate, α -D-lactose, fusidic acid, minocycline, and D-raffinose are not utilized as the sole source of carbon (Biolog GEN III).

Strain M1T305 (= NIBRBAC000499685) was isolated from the feces of a Siberian musk deer (*Moschus moschiferus*), Hwacheon, Gangwon Province, Korea. The DNA G+C content of the type strain is 35.9 mol%.

Description of *Streptococcus gallolyticus* subsp. *macedonicus* M1T307

Cells are Gram-staining positive. Colonies are circular, smooth, convex, and yellowish-white colored after 2 days of incubation on Tryptic soy agar (TSA) at 37°C. Dextrin, N-acetyl-D-glucosamine, D-fructose, D-galactose, L-rhamnose, 1% sodium lactate, acetoacetic acid, aztreonam, D-fucose, potassium tellurite, D-trehalose, D-cellobiose, gentiobiose, sucrose, D-turanose, D-salicin, sodium butyrate, D-mannitol, β -methyl-D-glucoside, N-acetyl- β -D-mannosamine, D-serine, glucuronamide, stachyose, D-melibiose, α-D-glucose, D-mannose, tetrazolium blue, vancomycin, tetrazolium violet, D-maltose, pectin, nalidixic acid, α -D-lactose, fusidic acid, and D-raffinose are utilized as the sole source of carbon. N-acetyl-D-galactosamine, inosine, myo-inositol, L-alanine, L-histidine, methyl pyruvate, lithium chloride, acetic acid, glycyl-L-proline, L-aspartic acid, L-glutamic acid, L-serine, L-lactic acid, D-malic acid, L-malic acid, bromosuccinic acid, propionic acid, L-fucose, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, D-arabitol, citric acid, γ-amino-butryric acid, glycerol, α -keto-butyric acid, D-gluconic acid, Tween 40, α -hydroxybutyric acid, 3-methyl glucose, D-glucose-6-phosphate, D-fructose-6-phosphate, N-acetyl neuraminic acid, D-aspartic acid, D-serine, gelatin, L-arginine, L-pyroglutamic acid, mucic acid, quinic acid, D-saccharic acid, D-lactic acid methyl ester, α -keto-glutaric acid, β -hydroxy-D, L-butyric acid, formic acid, rifamycin SV, guanidinium chloride, troleandomycin, lincomycin, D-sorbitol, niaproof 4, 4-hydroxyphenylacetic acid, sodium bromate, and minocycline are not utilized as the sole source of carbon (Biolog GEN III).

Strain M1T307 (=NIBRBAC000499688) was isolated from the feces of a Siberian musk deer (*Moschus moschiferus*), Hwacheon, Gangwon Province, Korea. The DNA G+C content of the type strain is 38.3 mol%.

Description of Rhodococcus phenolicus VT2414

Cells are Gram-staining positive, non-flagellated, and coccus-shaped. Colonies are circular, smooth, convex, and ivory colored after 2 days of incubation on TSA at

20°C. Sodium lactate, D-lactic acid, L-lactic acid, L-malic acid, succinic acid, Tween 40, potassium tellurite, β-butryric acid, acetoacetic acid, propionic acid, acetic acid, and sodium butyrate are utilized as the sole source of carbon (Biolog GEN III). Positive for following enzyme activities: reduction of nitrates, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase (API ZYM and API 20NE). Positive for the utilization of following substrates: valerate, 3-hydroxy-butyrate, suberate, acetate, D, L-lactate, and 3-hydroxy-benzoate (API ID 32 GN).

Strain VT2412 (= NIBRBAC000499827) was isolated from the feces of an Andean condor (*Vultur gryphus*), Gwacheon, Gyeonggi Province, Korea. The DNA G+C content of the type strain is 65.77 mol%.

Description of Brevibacterium siliguriense VT2418

Cells are Gram-staining positive, non-flagellated, and rod-shaped. Colonies are circular, smooth, convex, and ivory colored after 2 days of incubation on TSA at 20°C. *N*-glucosamin, *N*-galactosamin, α -D-glucose, D-fructose, D-galactose, D-fucose, L-fucose, inosine, sodium lactate, D-mannitol, D-arabitol, glycerol, D-glucose, D-fructose, D-aspartic acid, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, pyroglutamic acid, L-serine, D-gluconic acid, D-glucuronic acid, mucic acid, quinic acid, pheylacetic acid, D-lactic acid, L-lactic acid, citric acid, α-glutaric acid, D-malic acid, L-malic acid, succinic acid, nalidixic acid, potassium tellurite, Tween 40, γ -butryric acid, α -butryric acid, β -butryric acid, α -butyric acid, acetoacetic acid, propionic acid, acetic acid, aztreonam, and sodium butyrate are utilized as the sole source of carbon (Biolog GEN III). Positive for following enzyme activities: nitrate reduction, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, cystine arylamidase, trypsin and naphthol-AS-BI-phosphohydrolase (API ZYM and API 20NE). Positive for the utilization of following substrates: D-mannitol, D-glucose, propionate, valerate, citrate, L-histidine, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, D-ribose, suberate, acetate, D, L-lactate, L-alanine, glycogen, 3-hydroxy-benzoate, and L-serine (API ID 32 GN).

Strain VT2418 (= NIBRBAC000499828) was isolated from the feces of an Andean condor (*Vultur gryphus*), Gwacheon, Gyeonggi Province, Korea. The DNA G+C content of the type strain is 64.35 mol%.

Description of Glutamicibacter mysorens VT2504

Cells are Gram-staining positive, non-flagellated, and rod-shaped. Colonies are circular, smooth, convex, and ivory colored after 2 days of incubation on TSA at 20°C.

Dextrin, D-maltose, D-trehalose, D-cellobiolose, gentiobiose, sucrose, D-turanose, β -D-glucoside, D-salicin, α-D-glucose, D-mannose, D-fructose, D-galactose, inosine, sodium lactate, D-sorbitol, D-arabitol, glycerol, D-aspartic acid, gelatin, glycyl-L-proline, L-alanine, L-arginine, L-aspartic acid, L-glutamic acid, L-histidine, pyroglutamic acid, L-serine, D-gluconic acid, quinic acid, pheylacetic acid, L-lactic acid, citric acid, α-glutaric acid, L-malic acid, succinic acid, nalidixic acid, lithium chloride, potassium tellurite, Tween 40, β -butryric acid, acetoacetic acid, propionic acid, acetic acid, aztreonam, sodium butyrate, and sodium bromate are utilized as the sole source of carbon (Biolog GEN III). Positive for following enzyme activities: nitrate reduction, β -glucosidase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, naphthol-AS-BI-phosphohydrolase, α -glucosidase, β -glucosidase, and α -mannosidase (API ZYM and API 20NE). Positive for the utilization of following substrates: D-mannitol, D-glucose, D-sorbitol, L-arabinose, propionate, valerate, 3-hydroxy-butyrate, 4-hydroxy-benzoate, L-proline, L-rhamnose, D-ribose, D-sucrose, D-maltose, suberate, acetate, D, L-lactate, L-alanine, 5-ketogluconate, glycogen, 3-hydroxy-benzoate, and L-serine (API ID 32 GN).

Strain VT2504 (= NIBRBAC000499829) was isolated from the feces of an Andean condor (*Vultur gryphus*), Gwacheon, Gyeonggi Province, Korea. The DNA G+C content of the type strain is 60.35 mol%.

Description of Arthrobacter rhombi VM2501

Cells are Gram-staining positive, non-flagellated, and rod-shaped. Colonies are circular, smooth, convex, and pale-yellow colored after 2 days of incubation on MA at 20°C. D-maltose, D-trehalose, D-cellobiolose, gentiobiose, sucrose, D-turanose, neuraminic acid, α -D-glucose, sodium lactate, D-gluconic acid, nalidixic acid, sodium butyrate, and sodium bromate are utilized as the sole source of carbon (Biolog GEN III). Positive for following enzyme activities: nitrate reduction, glucose acidification, β -glucosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and β -glucosidase (API ZYM and API 20NE). Positive for the utilization of following substrates: D-mannitol, D-glucose, propionate, caprate, valerate, 3-hydroxy-butyrate, L-proline, D-ribose, D-sucrose, D-maltose, acetate, D, L-lactate, L-alanine, glycogen, and L-serine (API ID 32 GN).

Strain VM2501 (= NIBRBAC000499830) was isolated from the feces of an Andean condor (*Vultur gryphus*), Gwacheon, Gyeonggi Province, Korea. The DNA G+C content of the type strain is 67.90 mol%.

Description of Escherichia marmotae VM3408

Cells are Gram-staining positive, non-flagellated, and rod-shaped. Colonies are circular, smooth, convex, and ivory colored after 2 days of incubation on MA at 37°C. D-trehalose, D-melibiose, β -D-glucoside, N-glucosamine, β -mannosamin, N-galactosamin, neuraminic acid, D-mannose, D-fructose, D-galactose, L-fucose, L-rhamnose, inosine, sodium lactate, fusidic acid, D-sorbitol, glycerol, D-glucose, D-fructose, D-serine, troleandomycin, rifamycin SV, glycyl-L-proline, L-alanine, L-aspartic acid, L-glutamic acid, L-serine, lincomycin, guanidinium chloride, niaproof 4, galacturonic acid, L-galactonic lactone, D-gluconic acid, D-glucuronic acid, glucuronamide, mucic acid, D-saccharic acid, vancomycin, tetrazolium violet, tetrazolium blue, methyl pyruvate, L-lactic acid, α-glutaric acid, D-malic acid, L-malic acid, succinic acid, nalidixic acid, α -butryric acid, β -butryric acid, propionic acid, acetic acid, and sodium butyrate are utilized as the sole source of carbon (Biolog GEN III). Positive for following enzyme activities: nitrate reduction, indole production, glucose acidification, β -galactosidase, alkaline phosphatase, esterase (C4), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β -galactosidase, and α -glucosidase (API ZYM and API 20NE). Positive for the utilization of following substrates: D-mannitol, D-glucose, D-melibiose, L-fucose, D-sorbitol, L-arabinose, propionate, L-proline, L-rhamnose, N-acetyl-glucosamine, D-ribose, D-maltose, acetate, D, Llactate, L-alanine, and L-serine (API ID 32 GN).

Strain VM3408 (= NIBRBAC000499831) was isolated from the feces of an Andean condor (*Vultur gryphus*), Gwacheon, Gyeonggi Province, Korea. The DNA G+C content of the type strain is 48.49 mol%.

ACKNOWLEDGEMENTS

This work was supported by grants from the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR201601113, NIBR201701107 and NIBR201902111).

REFERENCES

Bäckhed, F., H. Ding, T. Wang, L.V. Hooper, G.Y. Koh, A. Nagy, C.F. Semenkovich and J.I. Gordon. 2004. The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the United States of America 101:15718-15723.

Bäckhed, F., R.E. Ley, J.L. Sonnenburg, D.A. Peterson and J.I. Gordon. 2005. Host-bacterial mutualism in the human

- intestine. 307:1915-1920.
- Baillie, J., C. Hilton-Taylor and S.N. Stuart. 2004. 2004 IUCN red list of threatened species: a global species assessment. (Iucn).
- Felsenstein, J. 1981. Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal of Molecular Evolution 17:368-376.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.
- Hall, T.A. 1999. BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series 41:95-98.
- Hoffmann, C., D.A. Hill, N. Minkah, T. Kirn, A. Troy, D. Artis and F. Bushman. 2009. Community-wide response of the gut microbiota to enteropathogenic Citrobacter rodentium infection revealed by deep sequencing. Infection and Immunity 77:4668-4678.
- Kluge, A.G. and F.S. Farris. 1969. Quantitative phyletics and the evolution of anurans. Systemic Zoology 18:1-32.
- 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.
- Lane, D.J. 1991. 16S/23S rRNA sequencing. In: E. Stackebrandt and M. Goodfellow (eds.), Nucleic acid techniques in bacterial systematics, John Wiley & Sons, New York. pp. 115-175.
- Round, J.L. and S.K. Mazmanian. 2009. The gut microbiota shapes intestinal immune responses during health and dis-

- ease. Nature Reviews Immunology 9:313-323.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Molecular Biology and Evolution 4:406-425.
- Stecher, B., R. Robbiani, A.W. Walker, A.M. Westendorf, M. Barthel, M. Kremer, S. Chaffron, A.J. Macpherson, J. Buer and J. Parkhill. 2007. Salmonella enterica serovar typhimurium exploits inflammation to compete with the intestinal microbiota. PLoS Biology 5:e244.
- Thompson, J.D., D.G. Higgins and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.
- Turnbaugh, P.J., R.E. Ley, M.A. Mahowald, V. Magrini, E.R. Mardis and J.I. Gordon. 2006. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 444:1027-1131.
- 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. International Journal of Systematic and Evolutionary Microbiology 67:1613-1617.

Submitted: October 25, 2019 Revised: January 6, 2020 Accepted: January 6, 2020