A report of 37 unrecorded anaerobic bacterial species isolated from the Geum River in South Korea

Changsu Lee, Joon Yong Kim, Yeon Bee Kim, Juseok Kim, Seung Woo Ahn, Hye Seon Song and Seong Woon Roh^*

Microbiology and Functionality Research Group, World Institute of Kimchi, Gwangju 61755, Republic of Korea

*Correspondent: seong18@gmail.com

A total of 37 anaerobic bacteria strains within the classes Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Bacteroidia, Flavobacteriia, Bacilli, Clostridia, and Fusobacteriia were isolated from freshwater and sediment of the Geum River in Korea. The unreported species were related with Rhizobium and Oleomonas of the class Alphaproteobacteria; Acidovorax, Pseudogulbenkiania, and Aromatoleum of the class Betaproteobacteria; Tolumonas, Aeromonas, Cronobacter, Lonsdalea, and Phytobacter of the class Gammaproteobacteria; Bacteroides, Dysgonomonas, Macellibacteroides, and Parabacteroides of the class Bacteroidia; Flavobacterium of the class Flavobacteriia; Bacillus and Paenibacillus of the class Bacilli; Clostridium, Clostridioides, Paraclostridium, Romboutsia, Sporacetigenium, and Terrisporobacter of the class Clostridia; and Cetobacterium and Ilyobacter of the class Fusobacteriia. A total of 37 strains, with >98.7% 16S rRNA gene sequence similarity with validly published bacterial species, but not reported in Korea, were determined to be unrecorded anaerobic bacterial species in Korea.

Keywords: 16S rRNA, anaerobic bacteria, bacterial diversity, taxonomy, unrecorded species

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INTRODUCTION

Since the Nagoya Protocol and the Convention on Biological Diversity, securing and managing of biological resources have become more important (Buck & Hamilton, 2011). In order to be competitive with national biological resources, various biological resources should be secured. However, to date, systematic research on domestic freshwater biological resources is insufficient. Freshwater basins are expected to have high diversity due to their diverse environmental conditions. According to the Korean Society of Ecology, reported in 1994, it is estimated that 100,000 native species inhabit Korea, while 52,628 species have been reported (National species list of Korea, 2019); so there is an urgent need to study unreported species. In particular, prokaryotes are a resource of biological industry and have the highest industrial value, but reported species are less than 1% of the estimated species. Most aerobic prokaryotes are being investigated among reported species. Although anaerobic prokaryotic microorganisms derived from freshwater environment are actively being studied globally due to their high novelty as biological resources, there are few cases of purely isolated culture in Korea.

In the present study, we attempted to isolate anaerobic microorganisms from freshwater and sediment in the Geum River of Korea. Here, 37 unreported species belonging to the classes *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Bacteroidia*, *Flavobacteriia*, *Bacilli*, *Clostridia*, and *Fusobacteriia* are reported and described.

MATERIALS AND METHODS

Freshwater and sediment samples were collected from urban streams and wetland of the Geum River watershed in 2019. A total of 37 anaerobic bacteria were isolated using various agar plates made of Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) medium No. 311c, DSMZ medium No. 320, DSMZ medium No. 1451, and reinforced clostridial medium. These agar plates were incubated at 15–25°C under anaerobic condition (BD GasPak EZ Anaerobe Pouch System) for 3–7 days. Isolated bacterial strains were purified by serial dilution spreading and the pure cells were preserved in 20% (v/v) glycerol suspension containing 10% skimmed milk at -80° C and as lyophilized ampoules.

Colony morphology of the strains was observed by eye or a magnifying glass after the cells were cultivated to their stationary phase on agar plates. Cellular morphology and cell size were examined by field emission transmission electron microscopy (JEM 2100F; Jeol). Growth in the presence of oxygen was tested by aerobic incubation for seven days. Gram staining was performed using a Gram-staining kit (BD). Biochemical characteristics were evaluated by using API 20NE (bioMérieux) according to the manufacturer's instructions.

Chromosomal DNA extraction, PCR amplification, and 16S rRNA gene sequencing were performed using standard procedures as described elsewhere (Kim *et al.*, 2019). For the determination of 16S rRNA gene sequences, primers 27F, 337F, 518R, 785F, and 1492R were used. Based on full 16S rRNA gene sequences, the closely related type species were obtained using the EzBioCloud server (Yoon *et al.*, 2017). 16S rRNA gene sequences were aligned with the most closely related strains using Clustal W (Thompson *et al.*, 1994). The phylogenetic trees were constructed using neighbor-joining (Saitou & Nei, 1987), maximum likelihood (Felsenstein, 1981), and maximum parsimony methods (Fitch, 1971) in MEGA7 (Kumar *et al.*, 2016) with bootstrap values based on 1,000 randomly generated trees.

RESULTS AND DISCUSSION

The designation of strains, ID, similarity, and source of isolation are described at Table 1. Thirty-seven strains were distributed into eight classes: two strains in Alphaproteobacteria, four strains in Bacilli, four strains in Bacteroidia, three strains in Betaproteobacteria, 16 strains in Clostridia, one strain in the Flavobacteriia, two strains in Fusobacteriia, and five strains in Gammaproteobacteria. Unrecorded anaerobic bacterial strains in the eight classes were identified as following species in the order of strain ID (Fig. 1): Clostridioides mangenotii, Clostridium botulinum, Clostridium lundense (Cirne et al., 2006), Pseudogulbenkiania subflava, Rhizobium alvei (Sheu et al., 2015), Sporacetigenium mesophilum (Chen et al., 2006), Terrisporobacter glycolicus (Collins et al., 1994), Clostridium huakuii (Ruan et al., 2014), Oleomonas sagaranensis, Clostridium algidicarnis, Clostridium intestinale (Collins et al., 1994), Paenibacillus sonchi (Hong et al., 2009), Paenibacillus riograndensis, Clostridium sartagoforme (Stackebrandt et al., 1999), Clostridium gasigenes (Broda et al., 2000), Romboutsia sedimentorum (Wang et al., 2015), Bacillus benzoevorans, Clostridium senegalense (Mishra et al., 2012), Clostridium aurantibutyricum, Acidovorax wautersii, Bacteroides luti, Flavobacterium tyrosinilyticum (Du & Yi, 2016), Lonsdalea britannica (Brady et al., 2012), Macellibacteroides fermentans (Jabari et al., 2012), Paraclostridium benzoelyticum (Tushar et al., 2015), Tolumonas auensis (Chertkov et al., 2011), Aeromonas rivipollensis (Marti & Balcázar, 2015), Cronobacter dublinensis subsp. lausannensis (Grim et al., 2013), Cetobacterium somerae (Finegold et al., 2003), Aromatoleum toluolicum (Krieger et al., 1999), Parabacteroides chartae (Tan et al., 2012), Dysgonomonas oryzarvi (Kodama et al., 2012), Clostridium amazonense (O'Neal et al., 2015), Clostridium chromiireducens (Inglett et al., 2011), Ilyobacter delafieldii, Phytobacter diazotrophicus (Zhang et al., 2008), and Bacillus endoradicis (Zhang et al., 2012).

Fourteen strains were isolated from freshwater and the others were isolated from sediment. Based on 16S rRNA gene sequences, phylogenetic position of 37 unrecorded strains is shown in Fig. 2. Detailed physiological and morphological characteristics of the 37 unrecorded bacterial strains determined in present study are given in the following strain descriptions.

Description of Clostridioides mangenotii CBA7501

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and cocci-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis and gelatinase. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7501 (=NNIBR2019644BA4) was isolated from a sediment sample, Jinan-gun, Jeollabuk-do, Korea.

Description of Clostridium botulinum CBA7502

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are irregular, raised, and entire after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7502 (=NNIBR2019644BA41) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

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C143.3	Order	ramuy	Cenus	opecies	otrain LU	UNIBK ID	number	Closest type strain	Accession number	(%)	source
Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Rhizobium	alvei	CBA7506	NNIBR2019644BA7	MN646970	TNR-22	HE649224	99.71	Freshwater
	Rhodospirillales	Acetobacteraceae	Oleomonas	sagaranensis	CBA7511	NNIBR2019644BA12	MN646975	HD-1	D45202	99.79	Freshwater
Bacilli	Bacillales	Bacillaceae	Bacillus	benzoevorans	CBA7520	NNIBR2019644BA20	MN646983	DSM 5391	D78311	99.64	Sediment
				endoradicis	CBA7541	NNIBR2019644BA42	MN971626	CCBAU 05776	GU434676	99.57	Sediment
		Paenibacillaceae	Paenibacillus	sonchi	CBA7514	NNIBR2019644BA15	MN646978	X19-5	DQ358736	99.39	Sediment
				riograndensis	CBA7516	NNIBR2019644BA16	MN646979	SBR5	LN831776	99.66	Sediment
Bacteroidia	Bacteroidales	Bacteroidaceae	Bacteroides	luti	CBA7524	NNIBR2019644BA24	MN646987	DSM 26991	jgi.1107761	99.24	Freshwater
		Dysgonamonadaceae	Dysgonomonas	oryzarvi	CBA7536	NNIBR2019644BA36	MN646999	Dy73	AB547446	99.79	Sediment
		Porphyromonadaceae	Macellibacteroides	fermentans	CBA7528	NNIBR2019644BA28	MN646991	LIND7H	HQ020488	99.45	Sediment
		Tannerellaceae	Parabacteroides	chartae	CBA7535	NNIBR2019644BA35	MN646998	NS31-3	JN029805	99.72	Sediment
Betaproteobacteria	Burkholderiales	Comamonadaceae	Acidovorax	wautersii	CBA7523	NNIBR2019644BA23	MN646986	DSM 27981	jgi.1068022	99.38	Freshwater
	Neisseriales	Chromobacteriaceae	Pseudogulbenkiania	subflava	CBA7505	NNIBR2019644BA6	MN646969	DSM 22618	FXAG01000045	99.66	Sediment
	Rhodocyclales	Rhodocyclaceae	Aromatoleum	toluolicum	CBA7534	NNIBR2019644BA34	MN646997	Т	AF129465	100.00	Sediment
Clostridia	Clostridiales	Clostridiaceae	Clostridium	botulinum	CBA7502	NNIBR2019644BA41	MN960557	Eklund 17B	CP001056	99.86	Sediment
				lundense	CBA7503	NNIBR2019644BA5	MN646968	DSM 17049	AY858804	99.51	Sediment
				huakuii	CBA7510	NNIBR2019644BA11	MN646974	LAM1030	KC967412	99.21	Freshwater
				algidicarnis	CBA7512	NNIBR2019644BA13	MN646976	DSM 15099	jgi.1107655	99.85	Sediment
				intestinale	CBA7513	NNIBR2019644BA14	MN646977	DSM 6191	X76740	99.49	Sediment
				sartagoforme	CBA7517	NNIBR2019644BA17	MN646980	DSM 1292	Y18175	99.86	Sediment
				gasigenes	CBA7518	NNIBR2019644BA18	MN646981	DSM 12272	AF092548	98.96	Freshwater
				senegalense	CBA7521	NNIBR2019644BA21	MN646984	JC122	JF824801	99.51	Sediment
				aurantibutyricum	CBA7522	NNIBR2019644BA22	MN646985	DSM 793	LZYW01000149	100.00	Sediment
				amazonense	CBA7537	NNIBR2019644BA37	MN647000	NE08V	KP281434	99.93	Freshwater
				chromüreducens	CBA7538	NNIBR2019644BA38	MN647001	GCAF-1	AY228334	98.74	Sediment
		Peptostreptococcaceae	Clostridioides	mangenotii	CBA7501	NNIBR2019644BA4	MN646967	DSM 1289	FR733662	99.02	Sediment
			Paraclostridium	benzoelyticum	CBA7529	NNIBR2019644BA29	MN646992	JC272	LBBT01000182	99.02	Sediment
			Romboutsia	sedimentorum	CBA7519	NNIBR2019644BA19	MN646982	LAM201	KF443808	99.93	Sediment
			Sporace tigenium	mesophilum	CBA7507	NNIBR2019644BA8	MN646971	ZLJ115	AY682207	99.44	Sediment
			Terrisporobacter	glycolicus	CBA7508	NNIBR2019644BA9	MN646972	DSM 1288	X76750	99.30	Freshwater
Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	tyrosinilyticum	CBA7526	NNIBR2019644BA26	MN646989	THGDN8.8	KR232271	99.35	Freshwater
Fusobacteriia	Fusobacteriales	Fusobacteriaceae	Cetobacterium	somerae	CBA7533	NNIBR2019644BA33	MN646996	WAL 14325	AJ438155	99.93	Freshwater
			llyobacter	delafieldii	CBA7539	NNIBR2019644BA39	MN647002	DSM 5704	FR733681	99.02	Sediment
Gammaproteobacteria Aeromonadales	t Aeromonadales	Aeromonadaceae	Tolumonas	auensis	CBA7530	NNIBR2019644BA30	MN646993	DSM 9187	CP001616	99.39	Sediment
			Aeromonas	rivipollensis	CBA7531	NNIBR2019644BA31	MN646994	P2G1	FR775967	99.72	Freshwater
	Enterobacterales	Enterobacteriaceae	Cronobacter	dublinensis subsp.	CBA7532	NNIBR2019644BA32	MN646995	LMG 23824	AJKY01000076	99.66	Freshwater
		Pectobacteriaceae	Lonsdalea	britannica	CBA7527	NNIBR2019644BA27	MN646990	LMG 26267	JF311442	99.55	Freshwater

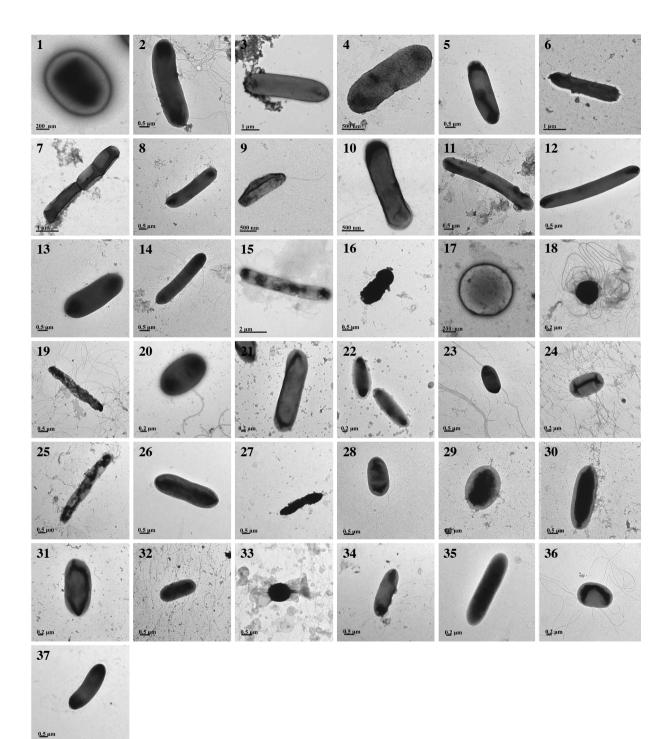


Fig. 1. Transmission electron micrographs of cells. Strains: 1, CBA7501; 2, CBA7502; 3, CBA7503; 4, CBA7505; 5, CBA7506; 6, CBA7507; 7, CBA7508; 8, CBA7510; 9, CBA7511; 10, CBA7512; 11, CBA7513; 12, CBA7514; 13, CBA7516; 14, CBA7517; 15, CBA7518; 16, CBA7519; 17, CBA7520; 18, CBA7521; 19, CBA7522; 20, CBA7523; 21, CBA7524; 22, CBA7526; 23, CBA7527; 24, CBA7528; 25, CBA7529; 26, CBA7530; 27, CBA7531; 28, CBA7532; 29, CBA7533; 30, CBA7534; 31, CBA7535; 32, CBA7536; 33, CBA7537; 34, CBA7538; 35, CBA7539; 36, CBA7540; 37, CBA7541.

Description of *Clostridium lundense* CBA7503

pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for glucose fermentation

Cells are obligate anaerobic, Gram-stain-positive, non-



Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between the strains isolated in this study. Bootstrap values (expressed as percentages of 1000 replications) of above 70% are shown at branch points. Filled circles and empty circles indicate nodes recovered by all three or two algorithms (neighbor-joining, maximum likelihood, and maximum parsimony), respectively. *Halolamina sediminis* halo-7^T (CVUA01000001) was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.

and esculin hydrolysis. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7503 (=NNIBR2019644BA5) was isolated from a sediment sample, Jinan-gun, Jeollabuk-do, Korea.

Description of Pseudogulbenkiania subflava CBA7505

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Utilizes D-glucose, D-maltose, and potassium gluconate. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7505 (=NNI-BR2019644BA6) was isolated from a sediment sample, Dong-gu, Daejeon, Korea.

Description of Rhizobium alvei CBA7506

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and curled after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7506 (=NNI-BR2019644BA7) was isolated from a freshwater sample, Buyeo-gun, Chungcheongnam-do, Korea.

Description of *Sporacetigenium mesophilum* CBA7507

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Does not utilize Dglucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7507 (=NNIBR2019644BA8) was isolated from a sediment sample, Buyeo-gun, Chungcheongnam-do, Korea.

Description of Terrisporobacter glycolicus CBA7508

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Utilize D-glucose. Does not utilize L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7508 (=NNIBR2019644BA9) was isolated from a freshwater sample, Jinan-gun, Jeollabuk-do, Korea.

Description of Clostridium huakuii CBA7510

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7510 (=NNIBR2019644BA11) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of Oleomonas sagaranensis CBA7511

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 320 at 25°C. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7511 (=NNIBR2019644BA12) was isolated from a freshwater sample, Gunsan-si, Jeollabuk-do, Korea.

Description of Clostridium algidicarnis CBA7512

Cells are facultative anaerobic, Gram-stain-positive, non-pigmented, and rod-shaped. Colonies are punctiform, convex, and lobate after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for esculin hydrolysis. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7512 (=NNIBR2019644BA13) was isolated from a sediment sample, Dong-gu, Daejeon, Korea.

Description of Clostridium intestinale CBA7513

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 1451 at 15°C. Positive for esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, and gelatinase. Utilizes D-glucose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, and malic acid. Does not utilize L-arabinose, potassium gluconate, D-maltose, capric acid, adipic acid, trisodium citrate, and phenylacetic acid. Strain CBA7513 (=NNIBR2019644BA14) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Paenibacillus sonchi CBA7514

Cells are facultative anaerobic, Gram-stain-positive, non-pigmented, and rod-shaped. Colonies are circular, umbonate, and entire after incubation for three days on DSMZ medium No. 1451 at 15°C. Positive for glucose fermentation and esculin hydrolysis. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Utilizes D-mannitol, potassium gluconate, D-maltose, and adipic acid. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7514 (=NNI-BR2019644BA15) was isolated from a sediment sample, Dong-gu, Daejeon, Korea.

Description of Paenibacillus riograndensis CBA7516

Cells are facultative anaerobic, Gram-stain-positive, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 1451 at 15°C. Positive for glucose fermentation, esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. Utilizes L-arabinose, *N*-acetyl-glucosamine, potassium gluconate, and D-maltose. Does not utilize D-glucose, D-mannose, D-mannitol, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7516 (=NNI-BR2019644BA16) was isolated from a sediment sample, Dong-gu, Daejeon, Korea.

Description of Clostridium sartagoforme CBA7517

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 1451 at 15°C. Positive for glucose fermentation and esculin hydrolysis. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Utilizes D-glucose, D-mannitol, N-acetyl-glucosamine, and D-maltose. Does not utilize L-arabinose, D-mannose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7517 (=NNI-BR2019644BA17) was isolated from a sediment sample, Buyeo-gun, Chungcheongnam-do, Korea.

Description of *Clostridium gasigenes* CBA7518

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 320 at 15°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Utilize D-mannitol. Does not utilize D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7518 (=NNIBR2019644BA18) was isolated from a freshwater sample, Buyeo-gun, Chungcheongnam-do, Korea.

Description of Romboutsia sedimentorum CBA7519

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are irregular, convex, and undulate after incubation for three days on DSMZ medium No. 1451 at 25°C. Positive for glucose fermentation. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7519 (=NNIBR2019644BA19) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Bacillus benzoevorans CBA7520

Cells are facultative anaerobic, Gram-stain-positive, non-pigmented, and cocci-shaped. Colonies are circular,

umbonate, and entire after incubation for three days on DSMZ medium No. 1451 at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Utilizes L-arabinose, D-mannitol, and adipic acid. Does not utilize D-glucose, D-mannose, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7520 (=NNIBR2019644BA20) was isolated from a sediment sample, Buyeo-gun, Chungcheongnam-do, Korea.

Description of Clostridium senegalense CBA7521

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and cocci-shaped. Colonies are irregular, convex, and undulate after incubation for four days on reinforced clostridial medium at 25°C. Positive for glucose fermentation and gelatinase. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7521 (=NNIBR2019644 BA21) was isolated from a sediment sample, Buyeo-gun, Chungcheongnam-do, Korea.

Description of *Clostridium aurantibutyricum* CBA7522

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for glucose fermentation, esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, and urease. Utilizes D-glucose and D-mannose. Does not utilize L-arabinose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7522 (=NNI-BR2019644BA22) was isolated from a sediment sample, Jinan-gun, Jeollabuk-do, Korea.

Description of Acidovorax wautersii CBA7523

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and short rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 320 at 25°C. Positive for nitrate reduction and glucose fermentation. Negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Utilizes D-mannitol, malic acid, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, and phenylacetic acid. Strain CBA7523 (=NNIBR2019644BA23) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of Bacteroides luti CBA7524

Cells are obligate anaerobic, Gram-stain-negative, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for seven days on DSMZ medium No. 311c at 25°C. Positive for β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7524 (=NNIBR2019644BA24) was isolated from a freshwater sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of *Flavobacterium tyrosinilyticum* CBA7526

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 320 at 25°C. Positive for glucose fermentation, esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. Utilize capric acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, D-maltose, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7526 (=NNI-BR2019644BA26) was isolated from a freshwater sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Lonsdalea britannica CBA7527

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for glucose fermentation and esculin hydrolysis. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Utilize capric acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, D-maltose, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7527 (=NNI-BR2019644BA27) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of *Macellibacteroides fermentans* CBA7528

Cells are facultative anaerobic, Gram-stain-positive, non-pigmented, and rod-shaped. Colonies are circular, umbonate, and entire after incubation for three days on DSMZ medium No. 1451 at 25°C. Positive for nitrate reduction, glucose fermentation, esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for indole production, arginine dihydrolase, and urease. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7528 (=NNI-BR2019644BA28) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of *Paraclostridium benzoelyticum* CBA7529

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are irregular, flat, and curled after incubation for three days on reinforced clostridial medium at 25°C. Positive for glucose fermentation. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7529 (=NNIBR2019644BA29) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Tolumonas auensis CBA7530

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 1451 at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7530 (=NNIBR2019644BA30) was isolated from a sediment sample, Dong-gu, Daejeon, Korea.

Description of Aeromonas rivipollensis CBA7531

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for glucose fermentation. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7531 (=NNI-BR2019644BA31) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of *Cronobacter dublinensis* subsp. *lausannensis* CBA7532

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, pulvinate, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for urease. Utilizes potassium gluconate, malic acid, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, D-maltose, capric acid, adipic acid, and phenylacetic acid. Strain CBA7532 (=NNI-BR2019644BA32) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of Cetobacterium somerae CBA7533

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and cocci-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 320 at 25°C. Positive for glucose fermentation, esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. Utilize capric acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7533 (=NNIBR2019644BA33) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of Aromatoleum toluolicum CBA7534

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 320 at 25°C. Positive for nitrate reduction and glucose fermentation. Negative for indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7534 (=NNIBR2019 644BA34) was isolated from a sediment sample, Donggu, Daejeon, Korea.

Description of Parabacteroides chartae CBA7535

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on reinforced clostridial medium at 25°C. Positive for glucose fermentation, esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7535 (=NNI-BR2019644BA35) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Dysgonomonas oryzarvi CBA7536

Cells are obligate anaerobic, Gram-stain-negative, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 311c at 25°C. Positive for glucose fermentation, esculin hydrolysis, and β -galactosidase activity. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, and gelatinase. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7536 (= NNIBR2019644BA36) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Clostridium amazonense CBA7537

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and cocci-shaped. Colonies are irregular, convex, and lobate after incubation for four days on reinforced clostridial medium at 25°C. Positive for β -galactosidase activity. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, and gelatinase. Utilize capric acid. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetyl-glucosamine, potassium gluconate, D-maltose, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7537 (=NNI-BR2019644BA37) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of Clostridium chromiireducens CBA7538

Cells are obligate anaerobic, Gram-stain-positive, non-

pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for four days on DSMZ medium No. 311c at 25°C. Positive for glucose fermentation. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7538 (= NNIBR2019644BA38) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

Description of Ilyobacter delafieldii CBA7539

Cells are obligate anaerobic, Gram-stain-positive, nonpigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 1451 at 25°C. Negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, esculin hydrolysis, gelatinase, and β -galactosidase activity. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-glucosamine, potassium gluconate, D-maltose, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7539 (=NNIBR2019644BA39) was isolated from a sediment sample, Dong-gu, Daejeon, Korea.

Description of Phytobacter diazotrophicus CBA7540

Cells are facultative anaerobic, Gram-stain-negative, non-pigmented, and short rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 1451 at 25°C. Positive for nitrate reduction, indole production, glucose fermentation, esculin hydrolysis, gelatinase, and β -galactosidase activity. Negative for arginine dihydrolase and urease. Utilizes D-mannitol, potassium gluconate, malic acid, and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, N-acetyl-glucosamine, D-maltose, capric acid, adipic acid, and phenylacetic acid. Strain CBA7540 (=NNIBR2019644BA40) was isolated from a freshwater sample, Dong-gu, Daejeon, Korea.

Description of Bacillus endoradicis CBA7541

Cells are facultative anaerobic, Gram-stain-positive, non-pigmented, and rod-shaped. Colonies are circular, convex, and entire after incubation for three days on DSMZ medium No. 320 at 25°C. Positive for glucose fermentation and esculin hydrolysis. Negative for nitrate reduction, indole production, arginine dihydrolase, urease, gelatinase, and β -galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, *N*-acetyl-glucosamine, and D-maltose. Does not utilize D-mannitol, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate, and phenylacetic acid. Strain CBA7541 (=NNI-BR2019644BA42) was isolated from a sediment sample, Jangsu-gun, Jeollabuk-do, Korea.

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