

Report on 30 unrecorded bacterial species of the phylum *Firmicutes* isolated from Korea in 2016

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During the course of investigation of indigenous prokaryotic species in Korea, a total of 30 bacterial strains belonging to the phylum *Firmicutes* were isolated from diverse environmental sites such as soil, avian feces, wastewater treatment plants, fermented vegetables, seawater, algae, sea cucumber, octopus and tidal flat sediment. Phylogenetic analysis based on 16S rRNA gene sequences revealed that each strain showed high sequence similarity ($\geq 98.7\%$) to the closest type strain and formed a robust phylogenetic clade with the most closely related species in the phylum *Firmicutes*. To date, there is no official record of these 30 species in Korea. Therefore, we report 26 species of 12 genera in the order *Bacillales* and 4 species of 4 genera in the order *Lactobacillales* which have not been reported in Korea. Morphological and biochemical characteristics, isolation sources and NIBR deposit numbers are described in the species descriptions.

Keywords: 16S rRNA, *Firmicutes*, indigenous Korean prokaryotic species, unreported species

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INTRODUCTION

Firmicutes is a widely distributed bacterial phylum that contains three classes: *Bacilli*, *Clostridia* and *Erysipelotrichia* (Ludwig *et al.*, 2009). Species of this phylum are generally known as Gram-positive, low G+C content containing rod/coccus bacteria that are found in diverse environmental habitats. *Firmicutes* can either be beneficiary or detrimental to the food- and beverage-related industries (Sakamoto and Konings, 2003; Teusink and Smid, 2006). Some evidence suggests that *Firmicutes* also has some significant effect on human and animal health (Carr *et al.*, 2002). Currently, species-specific identification method is largely used to detect food-spoilage related bacteria (Haakensen *et al.*, 2008).

During a major research program in 2016 that investigated indigenous prokaryotic species in Korea, envi-

ronmental samples from diverse habitats in Korea were collected and numerous novel and unrecorded bacteria were isolated. The purpose of the current study was to describe the bacterial species of the phylum *Firmicutes* which had not been previously reported in Korea. Here, we describe 30 unreported bacterial strains of the phylum *Firmicutes* that belong to sixteen genera within two orders.

MATERIALS AND METHODS

30 bacterial isolates were isolated from diverse environmental samples such as soil, avian feces, wastewater treatment plants, fermented vegetables, seawater, algae, sea cucumber, octopus and tidal flat sediment. Each environmental sample was handled separately and spread

on several culture media comprising R2A agar (BD), tryptic soy agar (TSA, BD), nutrient agar (NA, Conda), Marine agar (MA, BD), de Man, Rogosa and Sharpe Agar (MRSA, Merck) and MRSA supplemented with 3% (w/v) NaCl with pH 5.5. The isolates were incubated at 20-37°C for 1-5 days (Table 1). Briefly, the designated strain IDs, NIBR IDs, the highest 16S rRNA sequence similarities, isolation sources and incubation conditions are listed in Table 1. Pure cultures of the strains were isolated by repeated streaking and stored at -80°C with 10-30% glycerol suspension.

Cell morphology was determined by transmission electron microscopy. Gram staining was performed by following the standard procedures. Biochemical characterizations were analyzed using API 20NE galleries (bioMérieux) according to the manufacturer's protocols. Bacterial DNA extraction, PCR amplification and 16S rRNA gene sequencing were performed using standard procedures as described elsewhere (Lee and Cha, 2016). The 16S rRNA gene sequences of the strains assigned to the *Firmicutes* were compared with those of the type strains of validated bacterial species using the EzTaxon-e server (Kim *et al.*, 2012) using the multiple alignment program MUSCLE 3.8 (Edgar, 2004). Nucleotide substitution model test was based on the Akaike information criterion using jModelTest (Posada, 2008). Phylogenetic trees based on maximum-likelihood (Felsenstein, 1981) and neighbor-joining (Saitou and Nei, 1987) methods were reconstructed using the package phangorn in R (Schliep, 2011). Tree visualization was done in MEGA7.0 (Kumar *et al.*, 2016). Both tree topologies were evaluated based on bootstrap analysis of 1000 datasets (Felsenstein, 1985).

RESULTS AND DISCUSSION

30 strains were classified into two orders in the phylum *Firmicutes*; 26 strains in the order *Bacillales* and 4 strains in the order *Lactobacillales* (Table 1). These strains were Gram-staining-positive except for the strains LPB0128 and DMHB11. Cells of strain LPB0128 were shown to be exceptionally appendaged (Fig. 1). Colony size, morphology and physiological characteristics are presented in the species description section. In the order *Bacillales*, six strains were assigned to the family *Paenibacillaceae* within the genera *Brevibacillus* and *Paenibacillus*; 13 strains belonged to the family *Bacillaceae* in the genera *Bacillus*, *Oceanobacillus*, *Virgibacillus* and *Geobacillus*; four strains were assigned to the family *Planococcaceae* within the genera *Sporosarcina*, *Lysinibacillus*, *Paenisporosarcina* and *Rummeliibacillus*; two strains belonged to the family *Staphylococcaceae* in the genus *Staphylococcus*; and one strain

belonged to the family *Alicyclobacillaceae* in the genus *Tumebacillus* (Fig. 2). In the order *Lactobacillales*, one strain belonged to the family *Carnobacteriaceae* in the genus *Carnobacterium*; two strains were assigned to the family *Enterococcaceae* in the genera *Enterococcus* and *Vagococcus*; and one strain belonged to the family *Leuconostocaceae* in the genus *Leuconostoc* (Fig. 3). Here we report 30 bacterial species which have been unrecorded in Korea belonging to eight families in two orders of the phylum *Firmicutes*.

Description of *Brevibacillus brevis* HMF7750

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are flat, irregular and white-colored after 3 days of incubation on R2A at 30°C. Positive for nitrate reduction, hydrolysis of esculin and gelatin, but negative for indole production and fermentation of D-glucose, L-arginine and urea. Assimilates D-glucose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate and malate. Does not assimilate arabinose, mannose, caprate, adipate, trisodium citrate and phenylacetate. Strain HMF7750 (=NIBRBAC000498455) was isolated from soil.

Description of *Brevibacillus borstelensis* LPB0129

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, convex, entire and white-colored after 1 day of incubation on MA at 25°C. Positive for nitrate reduction and gelatin hydrolysis. Negative for indole production and esculin hydrolysis. N-Acetylglucosamine and malate are not assimilated. D-Glucose, L-arginine and urea are not fermented. β -Galactosidase activity is negative. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose, potassium gluconate, caprate, adipate, trisodium citrate and phenylacetate. Strain LPB0129 (=NIBRBAC000498519) was isolated from sea cucumber.

Description of *Brevibacillus laterosporus* PR22215

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are irregular, flat, glistening and cream-colored after 2 days of incubation on R2A at 20°C. Positive for nitrate reduction and hydrolysis of esculin and gelatin, but negative for indole production, fermentation of D-glucose, L-arginine and urea and β -galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, N-acetylglucosamine, D-maltose, malate and trisodium citrate. Does not utilize D-mannitol, potassium gluconate, caprate, adipate and phenylacetate. Strain PR22215 (=NIBRBAC000498400) was isolated from eagle feces.

Table 1. Summary of strains belonging to Firmicutes and their taxonomic affiliations.

Order	Family	Genus	Strain ID	NIBR ID	Most closely related species	Similarity (%)	Isolation source	Medium	Incubation condition		
	<i>Paenibacillaceae</i>	<i>Brevibacillus</i>	HMF7750	NIBRBAC000498455	<i>Brevibacillus brevis</i>	99.2	Soil	R2A	30°C, 3d		
		<i>Brevibacillus</i>	LPB0129	NIBRBAC000498519	<i>Brevibacillus borstelensis</i>	99.9	Sea cucumber	MA	25°C, 1d		
		<i>Brevibacillus</i>	PR22215	NIBRBAC000498400	<i>Brevibacillus laterosporus</i>	99.4	Eagle feces	R2A	20°C, 2d		
		<i>Paenibacillus</i>	AR23203	NIBRBAC000498389	<i>Paenibacillus chibensis</i>	98.9	Eagle feces	R2A	37°C, 2d		
		<i>Paenibacillus</i>	CF2	NIBRBAC000498558	<i>Paenibacillus amylolyticus</i>	98.9	Tidal flat sediment	R2A	30°C, 2d		
		<i>Paenibacillus</i>	BE2-15	NIBRBAC000498656	<i>Paenibacillus validus</i>	99.9	Tidal flat sediment	TSA	30°C, 5d		
<i>Bacillales</i>	<i>Bacillaceae</i>	<i>Bacillus</i>	ATS3402	NIBRBAC000498379	<i>Bacillus oceanisediminis</i>	98.9	Eagle feces	TSA	37°C, 2d		
		<i>Bacillus</i>	AR23201	NIBRBAC000498401	<i>Bacillus acidicer</i>	99.5	Blackfaced spoonbill feces	R2A	37°C, 1d		
		<i>Bacillus</i>	ATS2307	NIBRBAC000498403	<i>Bacillus mesophilum</i>	98.8	Blackfaced spoonbill feces	TSA	20°C, 2d		
		<i>Bacillus</i>	H18Y	NIBRBAC000498409	<i>Bacillus vietnamensis</i>	98.9	Wastewater treatment plant	R2A	30°C, 2d		
		<i>Bacillus</i>	LPB0160	NIBRBAC000498531	<i>Bacillus tianshenii</i>	99.0	Tidal flat sediment	MA	25°C, 1d		
		<i>Bacillus</i>	CAU 1.339	NIBRBAC000498505	<i>Bacillus berkeleyi</i>	99.0	Seawater	MA	30°C, 3d		
		<i>Bacillus</i>	BE2-30	NIBRBAC000498657	<i>Bacillus oleronius</i>	99.6	Tidal flat sediment	TSA	30°C, 5d		
		<i>Bacillus</i>	BE3-11	NIBRBAC000498658	<i>Bacillus shackletonii</i>	99.5	Tidal flat sediment	NA	30°C, 2d		
		<i>Bacillus</i>	DMHB11	NIBRBAC000498629	<i>Bacillus flexus</i>	99.9	Seawater	R2A	25°C, 3d		
		<i>Oceanobacillus</i>	JMW-27	NIBRBAC000498664	<i>Oceanobacillus lapialis</i>	99.6	Algae	MA	30°C, 4d		
		<i>Virgibacillus</i>	AR23209	NIBRBAC000498385	<i>Virgibacillus halophilus</i>	99.1	Eagle feces	R2A	37°C, 2d		
		<i>Virgibacillus</i>	AMA2302	NIBRBAC000498402	<i>Virgibacillus carmonensis</i>	99.1	Blackfaced spoonbill feces	MA	20°C, 2d		
		<i>Geobacillus</i>	NF3-3-1	NIBRBAC000498581	<i>Geobacillus kaustophilus</i>	99.3	Ginseng soil	R2A	25°C, 3d		
				<i>Sporosarcina</i>	PTS2210	NIBRBAC000498382	<i>Sporosarcina ureae</i>	99.3	Blackfaced spoonbill feces	TSA	20°C, 2d
		<i>Planococcaceae</i>	<i>Lysinibacillus</i>	LPB0128	NIBRBAC000498518	<i>Lysinibacillus massiliensis</i>	100	Sea cucumber	MA	25°C, 1d	
<i>Paenisporosarcina</i>	LPB0134		NIBRBAC000498522	<i>Paenisporosarcina quisquilarum</i>	99.0	Octopus	MA	25°C, 1d			
<i>Rummitibacillus</i>	AMR3201		NIBRBAC000498396	<i>Rummitibacillus stabeikisii</i>	99.9	Eagle feces	MRSA	37°C, 2d			
<i>Staphylococcaceae</i>	<i>Staphylococcus</i>	PR23219	NIBRBAC000498387	<i>Staphylococcus saprophyticus</i> subsp. <i>saprophyticus</i>	99.8	Blackfaced spoonbill feces	R2A	37°C, 2d			
	<i>Staphylococcus</i>	PR23304	NIBRBAC000498388	<i>Staphylococcus nepalensis</i>	99.9	Blackfaced spoonbill feces	R2A	37°C, 2d			
<i>Alicyclobacillaceae</i>	<i>Tumebacillus</i>	AR23202	NIBRBAC000498405	<i>Tumebacillus permanentifrigoris</i>	98.8	Blackfaced spoonbill feces	R2A,	37°C, 2d			
<i>Carnobacteriaceae</i>	<i>Carnobacterium</i>	PMA3404	NIBRBAC000498392	<i>Carnobacterium mobile</i>	99.5	Blackfaced spoonbill feces	MA	37°C, 3d			
<i>Lactobacillales</i>	<i>Enterococcaceae</i>	<i>Enterococcus</i>	AMR3203	NIBRBAC000498397	<i>Enterococcus casseliflavus</i>	99.7	Eagle feces	MRSA	37°C, 2d		
		<i>Vagococcus</i>	PR22212	NIBRBAC000498404	<i>Vagococcus carniphilus</i>	98.7	Eagle feces	R2A	20°C, 2d		
<i>Leuconostocaceae</i>	<i>Leuconostoc</i>	CAU 1.342	NIBRBAC000498498	<i>Leuconostoc mesenteroides</i> subsp. <i>mesenteroides</i>	99.9	Fermented vegetable	MRSA	30°C, 1d			

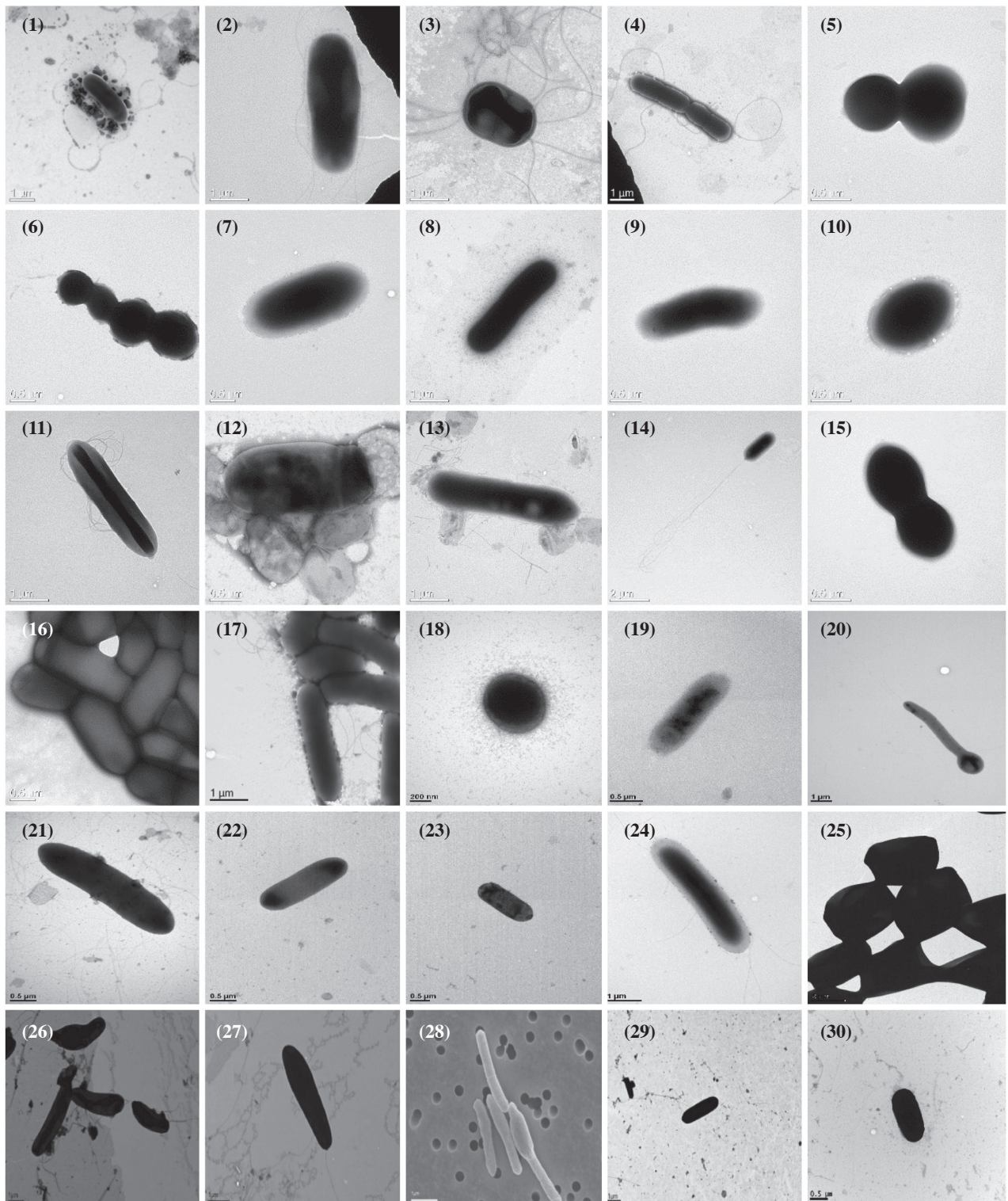


Fig. 1. Transmission electron micrographic and scanning electron micrographic images of isolated cells. Strains: 1, HMF7750; 2, ATS3402; 3, PTS2210; 4, AR23209; 5, PR23219; 6, PR23304; 7, AR23203; 8, PMA3404; 9, AMR3201; 10, AMR3203; 11, PR22215; 12, AR23201; 13, AMA2302; 14, ATS2307; 15, PR22212; 16, AR23202; 17, H18Y; 18, CAU 1342; 19, CAU 1339; 20, LPB0128; 21, LPB0129; 22, LPB0134; 23, LPB0160; 24, CF2; 25, NF3-3-1; 26, BE2-15; 27, BE2-30; 28, BE3-11; 29, JMW-27; 30, DMHB11.

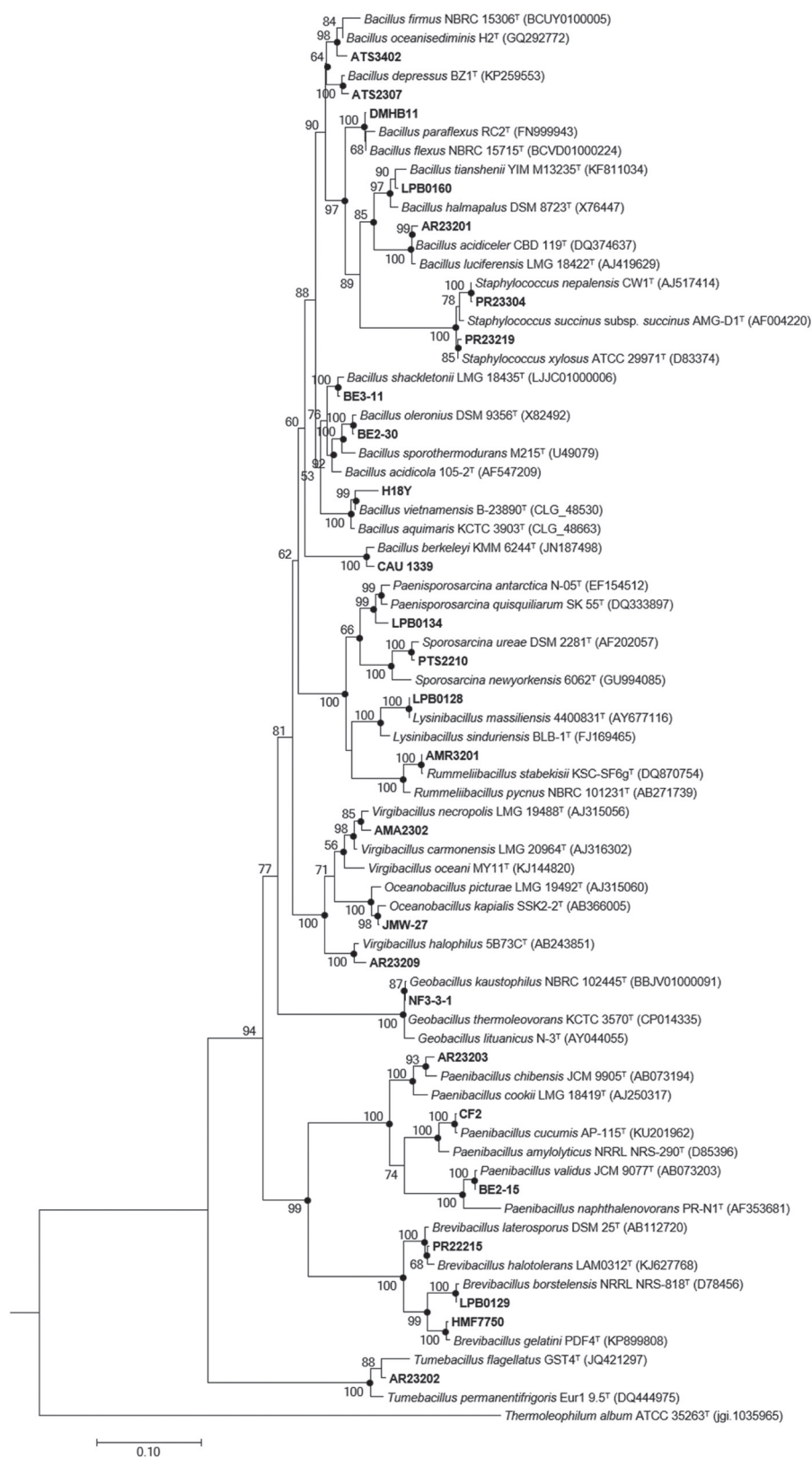


Fig. 2. Maximum-likelihood phylogenetic tree of isolates and related taxa belonging to the order *Bacillales*. Filled circles indicate the nodes that were recovered in the trees generated by both the neighbour-joining (NJ) and the maximum-likelihood (ML) methods. Numbers at nodes represent bootstrap values (NJ/ML) based on 1000 replicated datasets. Only values above 50% are shown. Bar, 0.1 substitutions per nucleotide position.

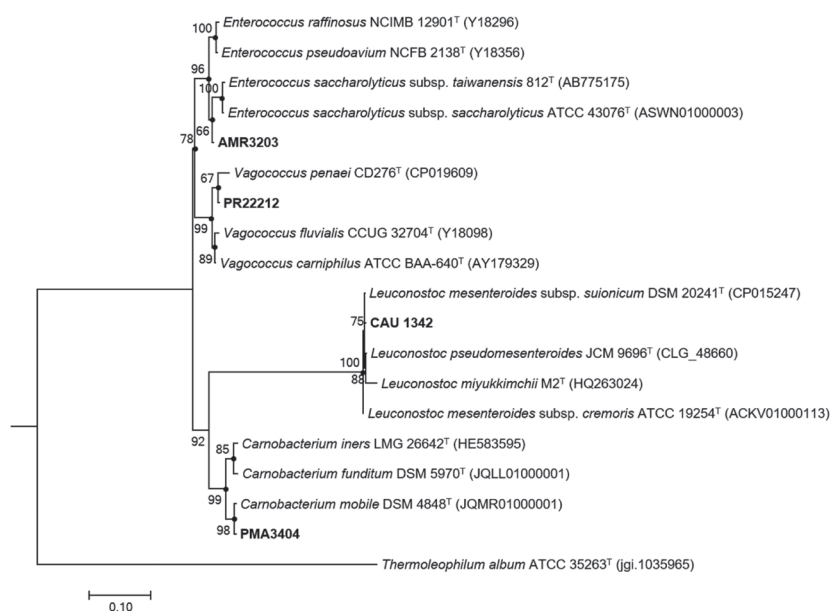


Fig. 3. Maximum-likelihood phylogenetic tree of isolates and related taxa belonging to the order *Lactobacillales*. Filled circles indicate the nodes that were recovered in the trees generated by both the neighbour-joining (NJ) and the maximum-likelihood (ML) methods and numbers at nodes represent bootstrap values (NJ/ML) based on 1000 replicated datasets. Only values above 50% are shown. Bar, 0.1 substitutions per nucleotide position.

Description of *Paenibacillus chibensis* AR23203

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are irregular and pale yellow-colored after 2 days of incubation on R2A at 37°C. Positive for nitrate reduction and esculin hydrolysis. Negative for indole production, fermentation of D-glucose, L-arginine and urea, gelatin hydrolysis and β -galactosidase activity. Assimilates D-glucose but does not assimilate L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain AR23203 (= NIBRBAC000498389) was isolated from eagle feces.

Description of *Paenibacillus amyolyticus* CF2

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are irregular, undulate, glistening and pale yellow-colored after 2 days of incubation on R2A at 30°C. Positive for nitrate reduction, hydrolysis of esculin and gelatin and β -galactosidase activity. Negative for indole production and fermentation of D-glucose, L-arginine and urea. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine and D-maltose. Does not utilize potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain CF2 (= NIBRBAC000498558) was isolated from tidal flat sediment.

Description of *Paenibacillus validus* BE2-15

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, umbonate, entire and cream-colored after 5 days of incubation on TSA at 30°C. Positive for esculin hydrolysis. Weakly positive for nitrate reduction. Negative for indole production, fermentation of D-glucose, L-arginine and urea, gelatin hydrolysis and β -galactosidase activity. Utilizes D-glucose, D-mannitol, D-maltose, potassium gluconate and malate. Utilization of D-mannose is weak. Does not utilize L-arabinose, *N*-acetylglucosamine, caprate, adipate, trisodium citrate and phenylacetate. Strain BE2-15 (= NIBRBAC000498656) was isolated from tidal flat sediment.

Description of *Bacillus oceanisediminis* ATS3402

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, flat and pale yellow-colored after 2 days of incubation on TSA at 37°C. Positive for nitrate reduction and urea fermentation, but negative for indole production, fermentation of D-glucose and L-arginine, hydrolysis of esculin and gelatin and β -galactosidase activity. Utilizes potassium gluconate and malate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, caprate, adipate, trisodium citrate and phenylacetate. Strain ATS3402 (= NIBRBAC000498379) was isolated from eagle feces.

Description of *Bacillus acidiceler* AR23201

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are irregular, filamentous, flat, glistening and white-colored after 1 day of incubation on R2A at 37°C. Positive for nitrate reduction and hydrolysis of esculin and gelatin. Negative for indole production, fermentation of D-glucose, L-arginine and urea and β -galactosidase activity. Utilizes D-glucose, D-mannitol, N-acetylglucosamine, D-maltose and potassium gluconate. Does not utilize L-arabinose, D-mannose, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain AR23201 (=NIBRBAC000498401) was isolated from blackfaced spoonbill feces.

Description of *Bacillus mesophilum* ATS2307

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, slightly convex, smooth, glistening and saffron-colored after 2 days of incubation on TSA at 20°C. Positive for nitrate reduction, esculin hydrolysis and β -galactosidase activity. Negative for indole production, fermentation of D-glucose, L-arginine and urea and gelatin hydrolysis. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, caprate, adipate, malate, trisodium citrate and phenylacetate but does not utilize potassium gluconate. Strain ATS2307 (=NIBRBAC000498403) was isolated from blackfaced spoonbill feces.

Description of *Bacillus vietnamensis* H18Y

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are irregular, smooth and pale yellow-colored after 2 days of incubation on R2A at 30°C. Positive for gelatin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, esculin hydrolysis and fermentation of D-glucose, L-arginine and urea. Utilizes D-glucose, D-mannose, D-mannitol and potassium gluconate. Does not utilize L-arabinose, N-acetylglucosamine, D-maltose, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain H18Y (=NIBRBAC000498409) was isolated from wastewater treatment plant.

Description of *Bacillus tianshenii* LPB0160

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and yellow-colored after 1 day of incubation on MA at 25°C. Positive for hydrolysis of esculin and gelatin. Negative for nitrate reduction, indole production, β -galactosidase activity and fermentation of D-glucose, L-arginine and urea. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodi-

um citrate and phenylacetate. Strain LPB0160 (=NIBRBAC000498531) was isolated from tidal flat sediment.

Description of *Bacillus berkeleyi* CAU 1339

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, sleek and glistening, convex and pale yellow-colored after 3 days of incubation on MA at 30°C. Positive for nitrate reduction and hydrolysis esculin and gelatin. Negative for indole production, fermentation of D-glucose, L-arginine and urea and β -galactosidase activity. Utilizes D-glucose, L-arabinose, D-maltose, adipate, malate and phenylacetate. Does not utilize D-mannose, D-mannitol, N-acetylglucosamine, potassium gluconate, caprate and citrate. Strain CAU 1339 (=NIBRBAC000498505) was isolated from seawater.

Description of *Bacillus oleronius* BE2-30

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular, convex, entire and cream-colored after 5 days of incubation on TSA at 30°C. Positive for nitrate reduction, but negative for indole production, fermentation of D-glucose, L-arginine and urea and β -galactosidase activity. Weakly hydrolyses esculin and gelatin. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain BE2-30 (=NIBRBAC000498657) was isolated from tidal flat sediment.

Description of *Bacillus shackletonii* BE3-11

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, lobate and cream-colored after 2 days of incubation on NA at 30°C. Positive for gelatin hydrolysis. Weakly positive for esculin hydrolysis and β -galactosidase activity. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain BE3-11 (=NIBRBAC000498658) was isolated from tidal flat sediment.

Description of *Bacillus flexus* DMHB11

Cells are Gram-staining-negative, flagellated and oval-shaped. Colonies are circular, slightly convex, opaque and white-colored after 3 days of incubation on R2A at 25°C. Positive for β -galactosidase activity. Negative for nitrate reduction, indole production, fermentation of D-glucose, L-arginine and urea and hydrolysis of esculin and gelatin. Utilizes D-glucose, L-arabinose, D-mannitol,

N-acetylglucosamine, D-maltose, potassium gluconate, malate and trisodium citrate. Strain DMHB11 (= NIBRBAC000498629) was isolated from seawater.

Description of *Oceanobacillus kapialis* JMW-27

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and cream-colored after 4 days of incubation on MA at 30°C. Positive for gelatin, but negative for nitrate reduction, indole production, fermentation of D-glucose, L-arginine and urea, esculin hydrolysis and β -galactosidase activity. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain JMW-27 (= NIBRBAC000498664) was isolated from algae.

Description of *Virgibacillus halophilus* AR23209

Cells are Gram-staining-positive, flagellated and rod or coccoid-shaped. Colonies are Circular, slightly convex and cream-colored after 2 days of incubation on R2A at 37°C. Positive for nitrate reduction and β -galactosidase activity, but negative for indole production, fermentation of D-glucose, L-arginine and urea and hydrolysis of esculin and gelatin. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain AR23209 (= NIBRBAC000498385) was isolated from eagle feces.

Description of *Virgibacillus carmonensis* AMA2302

Cells are Gram-staining-positive, flagellated and rod-shaped. Colonies are circular and pale pink-colored after 2 days of incubation on MA at 20°C. Positive for nitrate reduction, gelatin hydrolysis and β -galactosidase activity. Negative for indole production, fermentation of D-glucose, L-arginine and urea and esculin hydrolysis. Utilizes L-arabinose and potassium gluconate. Does not utilize D-glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain AMA2302 (= NIBRBAC000498402) was isolated from blackfaced spoonbill feces.

Description of *Geobacillus kaustophilus* NF3-3-1

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, slightly convex and pale yellow-colored after 3 days of incubation on R2A at 25°C. Positive for esculin hydrolysis, but negative for nitrate reduction, indole production, fermentation of D-glucose, L-arginine and urea, gelatin hydrolysis and

β -galactosidase activity. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine and potassium gluconate. Does not utilize D-maltose, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain NF3-3-1 (= NIBRBAC000498581) was isolated from ginseng soil.

Description of *Sporosarcina ureae* PTS2210

Cells are Gram-staining-positive, flagellated and rod or coccoid-shaped. Colonies are circular, smooth, glistering and saffron-colored after 2 days of incubation on TSA at 20°C. Positive for nitrate reduction, but negative for indole production, fermentation of D-glucose, L-arginine and urea, hydrolysis of esculin and gelatin and β -galactosidase activity. Utilizes malate and trisodium citrate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate and phenylacetate. Strain PTS2210 (= NIBRBAC000498382) was isolated from blackfaced spoonbill feces.

Description of *Lysinibacillus massiliensis* LPB0128

Cells are Gram-staining-negative, appendaged and rod-shaped. Colonies are circular, convex, entire and white-colored after 1 day of incubation on MA at 25°C. Positive for urea fermentation, but negative for nitrate reduction, indole production, fermentation of D-glucose and L-arginine, hydrolysis of esculin and gelatin and β -galactosidase activity. Utilizes D-mannitol and malate. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate and phenylacetate. Strain LPB0128 (= NIBRBAC000498518) was isolated from sea cucumber.

Description of *Paenisporosarcina quisquiliarum* LPB0134

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular, convex, entire and white-colored after 1 day of incubation on MA at 25°C. Positive for gelatin hydrolysis, but negative for nitrate reduction, indole production, fermentation of D-glucose and L-arginine, esculin hydrolysis and β -galactosidase activity. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain LPB0134 (= NIBRBAC000498522) was isolated from octopus.

Description of *Rummeliibacillus stabekisii* AMR3201

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and white-colored af-

ter 2 days of incubation on MRSA at 37°C. Positive for gelatin hydrolysis, but negative for nitrate reduction, indole production, fermentation of D-glucose, L-arginine and urea, esculin hydrolysis and β -galactosidase activity. Utilizes *N*-acetylglucosamine, potassium gluconate, adipate, malate, trisodium citrate and phenylacetate. Does not utilize D-glucose, L-arabinose, D-mannose, D-mannitol, D-maltose and caprate. Strain AMR3201 (= NIBRBAC000498396) was isolated from eagle feces.

Description of *Staphylococcus saprophyticus* subsp. *saprophyticus* PR23219

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and white-colored after 2 days of incubation on R2A at 37°C. Positive for nitrate reduction, urea fermentation and β -galactosidase activity. Negative for indole production, fermentation of D-glucose and L-arginine and hydrolysis of esculin and gelatin. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, malate and trisodium citrate. Does not utilize caprate, adipate and phenylacetate. Strain PR23219 (= NIBRBAC000498387) was isolated from blackfaced spoonbill feces.

Description of *Staphylococcus nepalensis* PR23304

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and white-colored after 2 days of incubation on R2A at 37°C. Positive for nitrate reduction, urea fermentation, esculin hydrolysis and β -galactosidase activity. Negative for indole production, fermentation of D-glucose and L-arginine and gelatin hydrolysis. Utilizes D-glucose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, adipate, malate, trisodium citrate and phenylacetate, but does not utilize L-arabinose and caprate. Strain PR23304 (= NIBRBAC000498388) was isolated from blackfaced spoonbill feces.

Description of *Tumebacillus permanentifrigoris* AR23202

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are irregular, flat and yellow-colored after 2 days of incubation on R2A at 37°C. Positive for nitrate reduction, esculin and gelatin hydrolysis and β -galactosidase activity. Negative for indole production and fermentation of D-glucose and L-arginine. Utilizes L-arabinose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, adipate and malate. Does not utilize D-glucose, D-mannose, caprate, trisodium citrate and phenylacetate. Strain AR23202 (= NIBRBAC000498405) was isolated from blackfaced spoonbill feces.

Description of *Carnobacterium mobile* PMA3404

Cells are Gram-staining-positive, non-flagellated and rod-shaped. Colonies are circular and cream-colored after 3 days of incubation on MA at 37°C. Positive for D-glucose fermentation, esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, fermentation of L-arginine and urea and gelatin hydrolysis. Utilizes D-glucose, *N*-acetylglucosamine and potassium gluconate. Does not utilize L-arabinose, D-mannose, D-mannitol, D-maltose, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain PMA3404 (= NIBRBAC000498392) was isolated from blackfaced spoonbill feces.

Description of *Enterococcus casseliflavus* AMR3203

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and white-colored after 2 days of incubation on MRSA at 37°C. Positive for D-glucose fermentation, esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, fermentation of L-arginine and urea and gelatin hydrolysis. Unable to utilize D-glucose, L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, D-maltose, potassium gluconate, caprate, adipate, malate, trisodium citrate and phenylacetate. Strain AMR3203 (= NIBRBAC000498397) was isolated from eagle feces.

Description of *Vagococcus carniphilus* PR22212

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular, glistening and white-colored after 2 days of incubation on R2A at 20°C. Positive for nitrate reduction and L-arginine fermentation, but negative for indole production, fermentation of D-glucose and urea, hydrolysis of esculin and gelatin and β -galactosidase activity. Utilizes D-mannitol, caprate, adipate and phenylacetate. Does not utilize D-glucose, L-arabinose, D-mannose, *N*-acetylglucosamine, D-maltose, potassium gluconate, malate and trisodium citrate. Strain PR22212 (= NIBRBAC000498404) was isolated from eagle feces.

Description of *Leuconostoc mesenteroides* subsp. *mesenteroides* CAU 1342

Cells are Gram-staining-positive, non-flagellated and coccoid-shaped. Colonies are circular and cream-colored after 1 day incubation in MRSA with 3% (w/v) NaCl and 5.5 pH at 30°C. Positive for D-glucose fermentation, esculin hydrolysis and β -galactosidase activity. Negative for nitrate reduction, indole production, fermentation of L-arginine and urea and gelatin hydrolysis. Utilizes D-glucose, L-arabinose, D-mannose, D-mannitol,

N-acetylglucosamine, D-maltose, potassium gluconate, adipate, malate, trisodium citrate and phenylacetate, but does not utilize caprate. Strain CAU 1342 (= NIBRBAC 000498498) was isolated from fermented vegetable.

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