Paenibacillus oceanisediminis sp. nov. isolated from marine sediment

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A Gram-stain-negative, non-motile, aerobic, endospore forming and rod-shaped bacterium, designated strain L10^T, was isolated from marine sediment collected from the South Korean coast. The organism grew optimally under conditions of 30 °C, 1 % (w/v) NaCl and pH 6.0. It was oxidase-negative and catalase-positive. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain L10^T was associated with the genus *Paenibacillus* and most closely related to *Paenibacillus barcinonensis* BP-23^T (98.2 % similarity). The major fatty acids of strain L10^T were iso-$C_{14:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. The cell-wall peptidoglycan was the A1γ type, and the predominant isoprenoid quinone was menaquinone-7. Strain L10^T contained two unidentified lipids, an unidentified amino-phospholipid, phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The G+C content of the genomic DNA was 44 mol% and the DNA–DNA hybridization values with closely related strains were below 14 ± 2 %. Based on phenotypic, genotypic, and phylogenetic data, strain L10^T should be classified as a novel species within the genus *Paenibacillus*. The name *Paenibacillus oceanisediminis* sp. nov. is proposed. The type strain is L10^T (=KACC 16203^T=JCM 17814^T).

The genus *Paenibacillus*, which belongs to the family *Paenibacillaceae*, was constructed by Ash *et al.* (1993, 1994). Based on the phylogenetic analysis of 16S rRNA gene sequences, the genus *Paenibacillus* was separated from the genus *Bacillus* for the rRNA group 3 bacilli. At the time of writing, 134 species of *Paenibacillus* have been described. The genus *Paenibacillus* is characterized as rod-shaped and endospore-forming bacteria, containing ansteiso-$C_{15:0}$ as the major fatty acid and a DNA G+C content of 39–54 mol% (Nakamura, 1984; Saha *et al.*, 2005). Members of the genus *Paenibacillus* are widely distributed in the environment, including in the air (Rivas *et al.*, 2005), cow faeces (Velázquez *et al.*, 2004), alkaline soil (Yoon *et al.*, 2005), and a fermented tea (Oh *et al.*, 2008), and play important roles in microbial population (Reva *et al.*, 1995). In this study, strain L10^T was identified as a novel species belonging to the genus *Paenibacillus*, based on evidence from physiological, biochemical, and genotypic investigations.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain L10^T is JF811909.

A supplementary figure is available with the online version of this paper.

Strain L10^T was isolated from a marine sediment sample collected from a cage-cultured ark clam farm in the Gangjin bay of Korea. The marine sediment sample was serially diluted with sterile PBS and spread on Luria–Bertani agar (LA; Difco), and incubated at 25 °C. The isolate was repeatedly restreaked on LA plates to obtain a pure culture. The 16S rRNA gene was amplified by colony PCR using a PCR pre-mix (WIZBIO) and two universal primers (8F and 1492R) (Baker *et al.*, 2003). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as previously described (Roh *et al.*, 2008). The results of the 16S rRNA gene sequence analysis showed that strain L10^T was very similar to *Paenibacillus barcinonensis* BP-23^T (98.2 % 16S rRNA gene sequence similarity), *Paenibacillus taichungensis* BCRC 17757^T (98.0 %), *Paenibacillus pabuli* JCM 9074^T (97.9 %), *Paenibacillus amylolyticus* NRRL NRS-290^T (97.4 %) and the type species of genus *Paenibacillus*, *Paenibacillus polymyxa* IAM 13419^T (93.3 %). The 16S rRNA gene sequences of strain L10^T and closely related species were aligned using the multiple sequence alignment program CLUSTAL W (Thompson *et al.*, 1994). The MEGA5 software program (Tamura *et al.*, 2011)

was used to determine the phylogenetic relationships of strain L10^T and closely related species using neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969), and maximum-likelihood (Felsenstein, 1981) methods with 1000 random bootstrap replicates, respectively. The neighbour-joining and maximum-likelihood phylogenetic analyses were performed using Kimura's two-parameter model with a gamma correction of 2. The maximum-parsimony phylogenetic analysis was implemented with Close-Neighbour-Interchange (CNI) on random trees, which was not heuristic. The results of the phylogenetic analysis showed that strain L10^T formed a cluster with closely related species in the genus *Paenibacillus*, including the type species of the genus *Paenibacillus*, and was most closely related to *P. barcinonensis* BP-23^T (Fig. 1). The 16S rRNA gene sequence of strain L10^T indicated high bootstrap values with closely related species in the genus *Paenibacillus*. To more comprehensively characterize strain L10^T, the type strains of four closely related species (based on 16S rRNA gene sequence similarity) and the type species of the genus *Paenibacillus* were selected for comparison and used as reference species: *P. barcinonensis* KCTC 13019^T, *P. pabuli* KCTC 3398^T, and *P. amylolyticus* KCTC 3455^T were obtained from the Korean Collection for Type Cultures (KCTC); *P. polymyxa* KACC 10485^T was obtained from the Korean Agricultural Culture Collection; and *P. taichungensis* DSM 19942^T was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ).

The temperature range and optimal temperature for growth of strain L10^T was determined by culture at different temperatures (4, 10, 15, 20, 25, 30, 37, 45, and 60 °C) in Luria–Bertani broth (LB; Difco), which was performed in triplicate. The growth of the type species of genus *Paenibacillus* at different temperatures (4 and 45 °C) was performed in LA medium for 7 days. Tolerance of various NaCl concentrations (0–11 % at intervals of 1 %, w/v) was tested using a medium that comprised all of the constituents of LB except NaCl, supplemented with appropriate concentrations of NaCl. Growth at different pH values (pH 4.0–11.0 at intervals of 1.0 pH unit) was tested on LB at 30 °C, in triplicate. The different pH values were adjusted with 10 mM MES (for pH 4, 5 and 6), 10 mM TAPS (for pH 7, 8 and 9) or 10 mM Na_2HPO_4 (for pH 10 and 11). The turbidity of each culture was measured as the optical density at 600 nm (OD_{600}) using a spectrophotometer (SYNERGY MX, BioTek) after 24 h, 48 h and 7 days incubation. Strain L10^T grew at 10–45 °C, 0–7 % (w/v) NaCl and at pH 5.0–10.0. The conditions for optimal growth of strain L10^T were 30 °C, 1 % (w/v) NaCl and pH 6.0. Unless stated otherwise, all tests characterizing strain L10^T were conducted under optimal growth

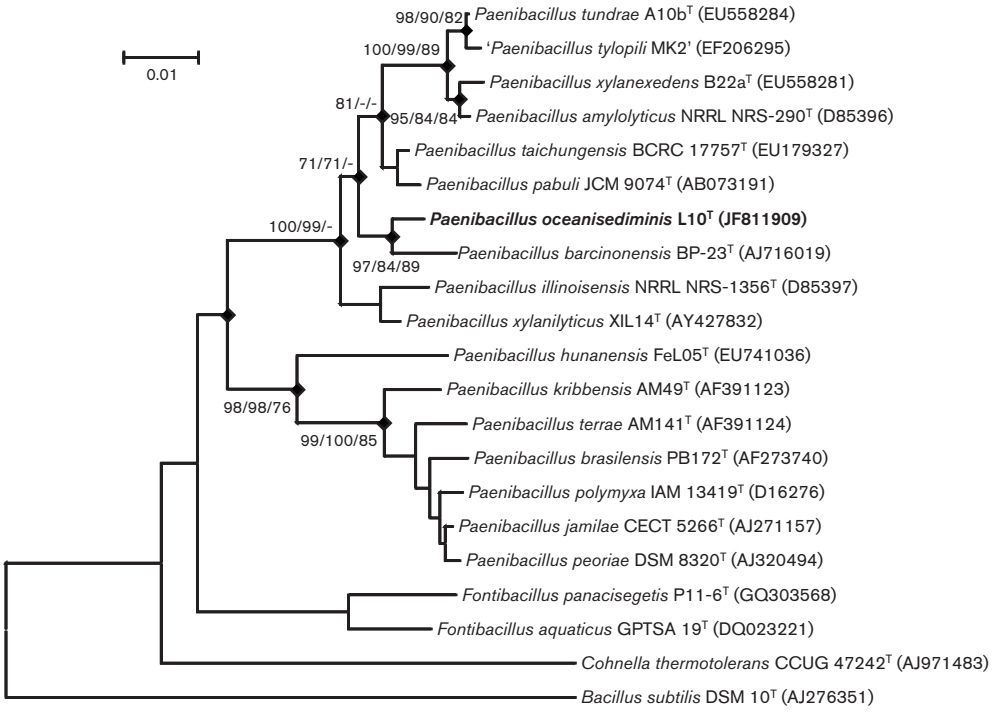

Fig. 1. Phylogenetic consensus tree based on 16S rRNA gene sequences showing the taxonomic positions of strain L10^T and type strains of closely related taxa. Numbers at nodes indicate bootstrap values (>70 %) from neighbour-joining/maximum-parsimony/maximum-likelihood methods as percentages of 1000 replicates. Filled diamonds represent genetic branches that were present in phylogenetic consensus trees generated by the three different methods. Bar, 0.01 accumulated changes per nucleotide.

Table 1. Comparison of the characteristics of strain L10^T and phylogenetic relatives in the genus *Paenibacillus*

Strains: 1, L10^T; 2, *P. barcinonensis* KCTC 13019^T (Sánchez *et al.*, 2005); 3, *P. taichungensis* DSM 19942^T (Lee *et al.*, 2008); 4, *P. pabuli* KCTC 3398^T (Nakamura, 1984); 5, *P. amylolyticus* KCTC 3455^T (Shida *et al.*, 1997); 6, *P. polymyxa* KACC 10485^T (Nanjo *et al.*, 2008). +, positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Gram reaction	−	+	Gram-variable	+	+	−
Growth at:		ND	ND	ND	ND	ND
5 °C	−	−	+	+	−	−*
45 °C	+	−	−	−	−	−*
5 % NaCl	+	+	+	−	−	−
DNA G+C content (mol%)	44	45	47	49	46−47	44−46
Acid production from:*	ND	ND	ND	ND	ND	ND
D-Arabinose	−	+	−	−	−	−
L-Xylose	+	−	−	+	−	−
L-Rhamnose	+	+	−	+	−	−
Inositol	−	+	−	+	−	−
D-Sorbitol	−	−	−	+	+	−
Methyl α-D-mannoside	+	+	−	−	+	−
N-Acetylglucosamine	+	+	+	+	+	−
Inulin	−	−	+	−	−	+
Melezitose	+	+	+	+	+	−
Glycogen	+	−	+	+	+	+
D-Tagatose	−	−	−	−	+	−
D-Fucose	−	−	−	−	+	−
L-Fucose	−	+	+	−	+	−
D-Arabitol	−	−	+	−	−	−
L-Arabitol	−	−	+	−	−	−
5-Ketogluconate	−	+	+	−	+	−
Enzyme activities:*	ND	ND	ND	ND	ND	ND
Trypsin	−	+	−	−	+	−
α-Chymotrypsin	−	+	+	+	+	+
Naphthol-AS-BI-phosphohydrolase	+	+	+	+	+	−
β-Glucuronidase	+	+	−	−	−	+
α-Mannosidase	−	−	−	−	+	−
α-Fucosidase	−	−	+	+	−	−
Utilization of:*	ND	ND	ND	ND	ND	ND
α-Cyclodextrin	+	−	−	−	+	−
Dextrin	+	+	+	−	+	+
Glycogen	−	−	−	−	−	+
N-Acetyl-D-glucosamine	+	−	−	−	−	−
L-Arabinose	+	−	−	−	−	+
D-Galactose	+	−	+	−	+	+
Gentiobiose	+	+	+	−	−	−
α-Lactose	+	−	+	+	+	+
Lactulose	+	−	+	−	+	+
D-Mannitol	+	−	+	−	−	+
D-Mannose	+	+	+	+	−	+
Melibiose	+	+	+	+	−	+
D-Psicose	+	+	+	−	+	+
D-Sorbitol	−	−	+	−	−	−
Turanose	+	−	+	−	+	+
Pyruvic acid methyl ester	+	−	−	−	+	+
Succinic acid monomethyl ester	+	−	−	−	+	−
Acetic acid	+	−	−	−	+	−
D-Gluconic acid	+	+	+	+	−	+
Succinamic acid	+	−	−	−	−	−
Inosine	+	+	+	−	+	+

Table 1. cont.

Characteristic	1	2	3	4	5	6
Uridine	+	+	+	−	+	+
Thymidine	+	+	+	−	+	+
2,3-Butanediol	−	−	−	−	−	+
D-Glucose 6-phosphate	−	−	+	−	−	−
Amygdalin†	ND	+	ND	+	+	ND
Arbutin†	ND	+	ND	+	+	ND
Maltotriose†	ND	+	ND	+	+	ND
Methyl α-D-galactoside†	ND	−	ND	+	−	ND
Methyl β-D-galactoside†	ND	+	ND	+	−	ND
Methyl α-D-glucoside†	ND	+	ND	−	+	ND
Palatinose†	ND	+	ND	−	+	ND
D-Ribose†	ND	+	ND	−	+	ND
Salicin†	ND	+	ND	+	+	ND
Pyruvic acid†	ND	+	ND	+	+	ND
Adenosine†	ND	+	ND	−	+	ND
β-Cyclodextrin†	ND	−	ND	−	+	ND
D-Xylose†	ND	−	ND	−	+	ND
2′-Deoxy adenosine†	ND	−	ND	−	+	ND
i-Erythritol‡	−	ND	+	ND	ND	−
cis-Aconitic acid‡	+	ND	−	ND	ND	−
Itaconic acid‡	+	ND	−	ND	ND	−
α-Ketobutyric acid‡	−	ND	+	ND	ND	−
L-Proline‡	+	ND	−	ND	ND	−

*Data of reference strains from this study.
†Data from GP2 MicroPlate (Biolog).
‡Data from GN2 MicroPlate (Biolog).

conditions. Morphological, physiological and biochemical analyses were performed with strain L10^T cultivated for 72 h at 30 °C in LB or LA medium. Gram staining was performed with a Gram staining kit (bioMérieux), following the manufacturer's instructions. Endospore formation was examined by the spore-staining method using malachite green, as described by Schaeffer & Fulton (1933). Gram- and spore-staining and cell morphology were observed using a light microscope (ECLIPSE 50*i*; Nikon). Motility was determined on semi-solid agar using the method of Tittsler & Sandholzer (1936). Catalase and oxidase activities were determined by bubble production in 3 % (v/v) hydrogen peroxide and the reactivity of 1 % (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux), respectively. API ZYM test strips (bioMérieux) were used to test the enzyme activities of strain L10^T according to the manufacturer's instructions. Utilization of sole carbon sources was tested using GN2 MicroPlates with GN/GP inoculating fluid (Biolog) with salinity adjusted to 2 % (w/v) NaCl, according to the manufacturer's instructions. Acid production from carbohydrates was tested using API 50CH test strips with 50 CHB/E medium (bioMérieux), following the manufacturer's instructions. Strain L10^T was Gram-stain-negative, endospore forming, non-motile, oxidase-negative, and catalase-positive. Endospore formation is a general characteristic of members of the family *Bacillaceae* (Logan *et al.*, 2009). The different biochemical characteristics of strain L10^T compared with the reference species are shown in Table 1.

Strain L10^T and the reference species were cultured on LA or in LB medium at 30 °C for 72 h, and then harvested for chemotaxonomic analyses. Physiological ages of strain L10^T and reference species were standardized as stationary phase. Fatty acids were extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999), analysed by gas chromatography (6890; Hewlett Packard), and then identified using the Microbial Identification software package (Sherlock version 4.0) (Sasser, 1990), which is based on the TSBA40 database. Isoprenoid quinones were extracted with chloroform/methanol (2 : 1, v/v), as described by Collins & Jones (1981a) and analysed by one-dimensional thin layer chromatography (TLC) on a silica gel 60 F_{254} plate (Merck), followed by reverse-phase high performance liquid chromatography (Collins & Jones, 1981b) using a Thermo ODS HYPERSIL (250 × 4.6 mm) column. Polar lipids were extracted according to the method of Xin *et al.* (2000) and separated using two-dimensional TLC on 20 cm^2 silica gel glass plates (Merck). Each spot on separate plates was analysed using appropriate detection reagents (Tindall, 1990), as follows: 10 % ethanolic molybdatophosphoric acid

reagent for total lipids, ninhydrin reagent for amino-containing lipids, Zinzadze reagent for phospholipids, and α-naphthol reagent for glycolipids. The composition of amino acids in the cell wall peptidoglycan was analysed using one-dimensional TLC on cellulose sheets (Bousfield *et al.*, 1985) with LL- and *meso*-diaminopimelic acid, alanine and glutamic acid as standard amino acids. The predominant cellular fatty acids (>10 %) of strain L10^T were iso-$C_{14:0}$, anteiso-$C_{15:0}$, and iso-$C_{16:0}$; complete fatty acid compositions of strain L10^T and the reference species are shown in Table 2. The predominant menaquinone of strain L10^T was MK-7, which was identical to those of the species in the genus *Paenibacillus*. The polar lipids of strain L10^T included two unidentified lipids, an unidentified amino-phospholipid, phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and diphosphatidylglycerol (DPG) (Fig. S1, available in IJSEM Online). PE, PG and DPG were also found in the type species of the genus *Paenibacillus* (Kämpfer *et al.*, 2006). Strain L10^T and the reference species possessed *meso*-diaminopimelic acid and alanine in the peptidoglycan amino acids, suggesting the presence of peptidoglycan type A1γ (Schleifer & Kandler, 1972).

Chromosomal DNA of strain L10^T and the reference species was extracted as described by Rochelle *et al.* (1992).

Table 2. Cellular fatty acid content (%) of strain L10^T and the reference species

Strains: 1, L10^T; 2, *P. barcinonensis* KCTC 13019^T; 3, *P. pabuli* KCTC 3398^T; 4, *P. taichungensis* DSM 19942^T; 5, *P. amylolyticus* KCTC 3455^T; 6, *P. polymyxa* KACC 10485^T. All data from this study. Values are percentages of total fatty acids. tr, Trace (<0.5 %); −, not detected.

Fatty acid	1	2	3	4	5	6
Saturated acids						
$C_{14:0}$	1.8	3.9	2.0	2.2	4.3	2.1
$C_{15:0}$	6.6	5.1	2.2	1.5	1.7	−
$C_{16:0}$	7.8	10.7	6.2	10.3	7.4	14.9
$C_{17:0}$	tr	tr	tr	tr	−	−
$C_{18:0}$	tr	0.7	tr	2.2	−	10.9
Unsaturated acids						
$C_{14:1}\omega5c$	−	−	−	−	−	1.5
$C_{16:1}\omega7c$ alcohol	tr	−	−	−	−	−
$C_{16:1}\omega11c$	0.5	tr	−	−	−	−
$C_{18:1}\omega7c$	−	0.5	0.5	1.0	−	−
$C_{18:1}\omega9c$	tr	0.8	0.5	0.8	−	1.9
Branched acids						
anteiso-$C_{13:0}$	tr	tr	tr	tr	tr	−
iso-$C_{14:0}$	10.1	13.9	8.0	10.3	16.9	2.0
iso-$C_{15:0}$	3.0	5.4	5.1	2.3	2.2	3.3
anteiso-$C_{15:0}$	45.6	39.9	55.3	49.0	49.3	34.4
iso-$C_{16:0}$	20.0	15.8	13.9	15.9	14.8	13.0
iso-$C_{17:0}$	0.9	0.9	1.3	0.8	tr	2.2
anteiso-$C_{17:0}$	2.6	1.5	3.4	3.2	1.8	10.3
$C_{16:0}$ N alcohol	−	−	−	−	−	2.4

The DNA G+C content was analysed using a fluorimetric method with SYBR Gold I and a real-time PCR thermocycler (Gonzalez & Saiz-Jimenez, 2002). The genomic DNA of *Escherichia coli* K-12, *Ruminococcus obeum* ATCC 29174^T, and *Ruegeria pomeroyi* DSS-3^T were used as calibration references. DNA–DNA hybridization was conducted using a genome-spotted microarray (Bae *et al.*, 2005; Chang *et al.*, 2008) with reciprocals to determine the genetic distances among strain L10^T and the reference species. DNA–DNA hybridization values were calculated by the signal-to-noise ratio (SNR) of each probe with the formula of Loy *et al.* (2005). The DNA G+C content of strain L10^T was 44 mol%, which lies in the range of other species in the genus *Paenibacillus*. The DNA–DNA hybridization values for strain L10^T with *P. barcinonensis* KCTC 13019^T, *P. taichungensis* DSM 19942^T, *P. pabuli* KCTC 3398^T and *P. amylolyticus* KCTC 3455^T were 14±2 %, 5±1 %, 5±1 % and 13±2 %, respectively. Strain L10^T was considered to be a distinct genospecies, since the DNA–DNA hybridization values were below the threshold of 70 % (Wayne *et al.*, 1987).

Based on the results of phenotypic, genotypic, and phylogenetic analyses, strain L10^T is classified as a novel species of the genus *Paenibacillus*, and the name *Paenibacillus oceanisediminis* sp. nov. is proposed.

Description of *Paenibacillus oceanisediminis* sp. nov.

Paenibacillus oceanisediminis (o.ce.a.ni.se.di′mi.nis. L. n. *oceanus* the great sea; L. n. *sedimen -inis* a sediment; N.L. gen. n. *oceanisediminis* of a sediment of the sea).

Cells are Gram-stain negative, non-motile, endospore forming, aerobic and rod-shaped (0.3–0.5 μm long and 1.0–2.3 μm wide). Spherical endospores are formed in a terminal position. Colonies on LA medium are circular, 0.1–0.5 mm in diameter, yellow with an entire margin, after incubation for 3 days at 30 °C. Growth occurs at a temperature range of 10–45 °C (optimum, 30 °C), in 0–7 % (w/v) NaCl (optimum, 1 %) and in the pH range of 5.0–10.0 (optimum, pH 6.0). Strain L10^T is catalase-positive and oxidase-negative. Based on the API ZYM test strip results, strain L10^T is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucosidase, leucine arylamidase, β-glucuronidase, and α-glucosidase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase. On the GN2 MicroPlate, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-lactose, lactulose, D-mannose, melibiose, sucrose, trehalose, succinic acid mono-methyl-ester, inosine, uridine, thymidine, glycerol, α-cyclodextrin, dextrin, *N*-acetyl-D-glucosamine, L-arabinose, maltose, D-mannitol, methyl β-D-glucoside, D-psicose, raffinose, turanose, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, D-gluconic acid, itaconic acid,

succinamic acid, and L-proline are utilized for growth. Acids are produced from glycerol, L-arabinose, D-ribose, D-xylose, L-xylose, methyl β-D-xyloside, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, D-mannitol, methyl α-D-mannoside, methyl α-D-glucoside, *N*-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, melibiose, sucrose, trehalose, melezitose, raffinose, starch, glycogen, gentiobiose, turanose and gluconate on API 50CHB/E strips. The dominant cellular fatty acids are iso-$C_{14:0}$, anteiso-$C_{15:0}$, and iso-$C_{16:0}$. The predominant menaquinone is MK-7. The polar lipid profile contained unidentified lipids, an unidentified amino-phospholipid, phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. The peptidoglycan cell wall is A1γ type.

The type strain is L10^T (=KACC 16203^T=JCM 17814^T), which was isolated from marine sediment collected from the South Korean coast. The DNA G+C content of the type strain is 44 mol%.

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