Halobacillus profundus sp. nov. and *Halobacillus kuroshimensis* sp. nov., moderately halophilic bacteria isolated from a deep-sea methane cold seep

Ngoc-Phuc Hua,¹ Atsuko Kanekiyo,¹ Katsunori Fujikura,^{1,2} Hisato Yasuda³ and Takeshi Naganuma¹

Correspondence
Takeshi Naganuma
takn@hiroshima-u.ac.jp

¹Graduate School of Biosphere Science, Hiroshima University, Kagamiyama, Higashi-Hiroshima 739-8528, Japan

²Japan Agency for Marine-Earth Science and Technology, Natsushima-cho, Yokosuka 237-0061, Japan

³Center for Advanced Marine Core Research, Kochi University, Monobe, Nankoku 783-8502, Japan

Two Gram-positive, rod-shaped, moderately halophilic bacteria were isolated from a deep-sea carbonate rock at a methane cold seep in Kuroshima Knoll, Japan. These bacteria, strains IS-Hb4^T and IS-Hb7^T, were spore-forming and non-motile. They were able to grow at temperatures as low as 9 °C and hydrostatic pressures up to 30 MPa. Based on high sequence similarity of their 16S rRNA genes to those of type strains of the genus *Halobacillus*, from 96.4 % (strain IS-Hb7^T to *Halobacillus halophilus* NCIMB 9251^T) to 99.4 % (strain IS-Hb4^T to *Halobacillus dabanensis* D-8^T), the strains were shown to belong to this genus. DNA–DNA relatedness values of 49.5 % and 1.0–33.0 %, respectively, were determined between strains IS-Hb4^T and IS-Hb7^T and between these strains and other *Halobacillus* type strains. Both strains showed the major menaquinone MK7 and L-Orn–D-Asp cell-wall peptidoglycan type. Straight-chain C_{16:0}, unsaturated C_{16:1ω7c} alcohol and C_{18:1ω7c} and cyclopropane C_{19:0} cyc fatty acids were predominant in both strains. The DNA G + C contents of IS-Hb4^T and IS-Hb7^T were respectively 43.3 and 42.1 mol%. Physiological and biochemical analyses combined with DNA–DNA hybridization results allowed us to place strains IS-Hb4^T (= JCM 14154^T = DSM 18394^T) and IS-Hb7^T (= JCM 14155^T = DSM 18393^T) in the genus *Halobacillus* as the respective type strains of the novel species *Halobacillus profundus* sp. nov. and *Halobacillus kuroshimensis* sp. nov.

The genus *Halobacillus*, with the type species *Halobacillus halophilus*, was established and described by Spring and co-workers in 1996 (Claus *et al.*, 1983; Spring *et al.*, 1996). So far, nine species of this genus have been identified (Amoozegar *et al.*, 2003a, b; Yoon *et al.*, 2003, 2004, 2005; Liu *et al.*, 2005). The increasing number of publications on bio-applications and other aspects of *Halobacillus* and the large number of 16S rRNA gene sequences deposited in databases for unidentified species reflect the wide distribution of these bacteria and their considerable scientific

interest (Burja *et al.*, 1999; Pinar *et al.*, 2001; Yang *et al.*, 2002; Rivadeneyra *et al.*, 2004). In our investigations of the diversity of halophilic bacteria in various habitats over the last few years, we obtained strains IS-Hb4^T and IS-Hb7^T in an endemic population from a carbonate rock collected at a deep-sea methane seep at Kuroshima Knoll, south-western Japan. Living in the special environment of low temperature, high pressure and low oxygen availability, these bacteria possess several features that are different from those of described species that would be considered evidence of interesting adaptation.

Samples from which the bacteria were isolated, carbonate rocks authentically formed in a methane seep of the Kuroshima Knoll, located at 642 m depth at 24° 07.8' N 124° 11.2' E (Fujikura *et al.*, 2003; Inagaki *et al.*, 2004), were collected with the remotely operated vehicle *Dolphin 3K*, operated by the Japan Agency for Marine Earth Science and

Abbreviation: CZE, capillary zone electrophoresis.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains IS-Hb4^T and IS-Hb7^T are AB189298 and AB195680.

Genetic relatedness of strains IS-Hb4^T and IS-Hb7^T to related *Halobacillus* type strains and differential carbon source utilization patterns are available with the online version of this paper.

Technology (JAMSTEC). The *in situ* water temperature, salinity (electric conductivity) and methane concentration were 7.6 °C, 35.5 mS cm⁻¹ and ~1 µM. An enrichment medium containing 0.5 % (w/v) Bacto-peptone (Difco), 0.25 % yeast extract (Difco), 0.1 % D-glucose (Wako) and 15 % NaCl (Okamoto *et al.*, 2001; Okamoto & Naganuma, 2003; Okamoto, 2004) in distilled water was used to culture and store the new isolates at 30 °C, except for certain changes in salinity and temperature in some tests. Distinct single colonies on 1.5 % agar plates of the generic medium were selected and isolated at least three times to obtain pure cultures. Type strains *Halobacillus halophilus* DSM 2266^T, *Halobacillus litoralis* DSM 10405^T, *Halobacillus salinus* JCM 11546^T, *Halobacillus trueperi* DSM 10404^T, *Halobacillus karajensis* DSM 14948^T, *Halobacillus localis* DSM 16468^T, *Halobacillus yeomjeoni* DSM 17110^T, *Halobacillus aidingensis* DSM 18198^T and *Halobacillus dabanensis* DSM 18199^T were used as reference strains.

Gram reaction, acid-fast staining, motility and endospore observation, enzyme activities (catalase, urease, phenylalanine deaminase, oxidase) and hydrolysis of starch, aesculin, gelatin and casein were tested according to conventional protocols (Doetsch, 1981; Smibert & Krieg, 1981). For anaerobic growth and nitrate reduction examination, the bacteria were tested using the same medium in which they grew well in isolation step both on agar plates and in liquid broth in an anaerobic atmosphere of (v/v) 80 % N₂, 10 % CO₂ and 10 % H₂ (Te-Her Anaero-Box ANX-1 system; Hirasawa). Media (in flasks for agar culture and in test tubes for liquid culture) were well gassed under nitrogen to remove oxygen before being autoclaved. Test tubes and agar plates were manipulated inside the anaerobic chamber. Any residual oxygen carried over or generated unexpectedly by the culture procedure inside the chamber was detected by an oxygen sensor and eliminated by the system until undetectable. Colonies and OD₆₀₀ were observed to determine anaerobic growth. Liquid medium supplemented with 0.1 % KNO₃ in test tubes containing Durham tubes was inoculated aerobically and anaerobically for nitrate reduction assay. *Escherichia coli* K-12 and *Virgibacillus marismortui* DSM 12325^T were used as positive controls and *H. litoralis* DSM 10405^T, *H. trueperi* DSM 10404^T and *H. salinus* JCM 11546^T were used as negative controls; uninoculated medium was also included. Sulfanilic acid and α-naphthylamine solutions and zinc powder were used to detect formation of nitrite and denitrification as described in the conventional method (Smibert & Krieg, 1981). Ability to utilize single carbon sources was tested using Biolog microplates based on the instructions of the manufacturer and previous studies (Garland & Mills, 1991; Garland, 1996, 1999). Acid production from carbohydrates was assayed using API 50CH and API 20 CE kits (bioMérieux). Aerobic growth at hydrostatic pressures of 0.1, 10, 20 and 30 MPa was examined using stainless-steel pressure vessels (Rigosha) compressed with a high-pressure stainless steel hand pump model 11-100 equipped with a pressure gauge (Enerpac). Inoculated liquid medium with 10 % NaCl added was filled

into sterile polyethylene transfer pipettes. The pipettes were then heat-sealed with complete exclusion of air bubbles and incubated in the pressure vessels under a certain pressure at room temperature for 1 week. Bacterial growth was observed as turbidity or formation of cell clumps.

Bacterial cells from cultures at stationary phase were harvested and genomic DNA was extracted according to protocol described by Wilson (1995). The PCR procedure of DeLong (1992) for amplifying the 16S rRNA gene was followed. Nearly full-length sequences (~1500 bp) of amplified 16S rRNA genes were obtained using a Dual CyDye Terminator sequencing kit (Amersham Biosciences) and sequenced with a Long-Read Tower sequencer (Visible Genetics). The sequences obtained were used to search for similarity on public databases (DDBJ/GenBank/EMBL) using the FASTA program (Pearson & Lipman, 1988) and aligned with CLUSTAL X software version 1.83 (Thompson *et al.*, 1997). Phylogenetic trees were then constructed based on neighbour-joining, minimum evolution and maximum-parsimony methods using MEGA 3.1 (Kumar *et al.*, 2004) and compared with those inferred by using the SEQBOOT, DNADIST, DNAMLK and CONSENSE programs of the PHYLIP package version 3.6 (Felsenstein, 2004) through 100–1000 bootstrap replications. Rooted trees which showed only minor differences in topology and high frequency of bootstrapping were chosen for phylogenetic analysis.

Non-radioactive digoxigenin-labelling DNA hybridization and anti-digoxigenin chemiluminescent detection methods were applied to determine DNA–DNA relatedness of the novel strains to all type strains of the genus *Halobacillus*. DIG-11-dUTP-labelled ssDNAs were hybridized with reference ssDNAs immobilized on positively charged nylon filter membrane (Brown, 1995) using DIG-High Prime DNA Labelling, Detection Starter kit II and DIG Wash and Block buffer set (Roche Molecular Biochemicals) under the manufacturer's instructions. The chemiluminescent density of hybrids was detected using the VersaDoc Imaging System model 5000 and analysed with Quantity One software version 4.4 (Bio-Rad). Experiments were repeated three times. The G + C content of the strains was determined by capillary zone electrophoresis (CZE) (Fraga *et al.*, 2002; Hua & Naganuma, 2007). Highly purified genomic DNAs were enzymically hydrolysed into nucleosides (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989) and nucleosides were then eluted in an alkaline phosphate buffer system, separated and detected quantitatively by a CAPI-3300 multichannel CZE (Otsuka Electronics). Cellular fatty acids were extracted and analysed using conventional GC-MS methods (Sasser, 2001). Quinones were extracted from cells according to Nishijima *et al.* (1997) and analysed by HPLC. Bacterial cell walls were hydrolysed and peptidoglycans were extracted and purified using high-performance TLC. The amino acid composition of hydrolysed peptidoglycans was detected using HPLC (Komagata & Suzuki, 1987).

The 16S rRNA gene sequences of strains IS-Hb4^T and IS-Hb7^T showed high similarity to those of type strains of genus *Halobacillus*, *H. halophilus* NCIMB 9251^T (97.3 and 96.4 %, respectively), *H. trueperi* DSM 10404^T (98.9 and 97.9 %), *H. litoralis* SL-4^T (98.8 and 97.8 %), *H. karajensis* DSM 14948^T (99.2 and 98.1 %), *H. salinus* HSL-3^T (97.8 and 97.0 %), *H. locisalis* SL-4^T (98.3 and 97.6 %), *H. yeomjeoni* MSS-402^T (98.2 and 97.7 %), *H. ainingensis* AD-6^T (98.0 and 97.2 %) and *H. dabanensis* D-8^T (99.4 and 98.5 %) (see Supplementary Table S1 in IJSEM Online), confirming their close relationship at the species level in this genus. Phylogenetic relationships of strains IS-Hb4^T and IS-Hb7^T and other type strains as well as other species of bacilli are shown in Fig. 1. The novel strains shared low levels of DNA–DNA relatedness with *H. halophilus* DSM 2266^T (12.2 and 30.7 % relatedness, respectively, with IS-Hb4^T and IS-Hb7^T), *H. trueperi* DSM 10404^T (33.0 and 20.1 %), *H. litoralis* DSM 10405^T (24.6 and 14.7 %), *H. karajensis* DSM 14948^T (10.4 and 27.4 %), *H. salinus* JCM 11546^T (25.4 and 18.6 %), *H. locisalis* (8.5 and 23.5 %), *H. yeomjeoni* DSM 17110^T (1.0 and 1.4 %), *H. ainingensis* DSM 18198^T (17.1 and 23.4 %) and *H. dabanensis* DSM 18199^T (6.0 and 5.3 %) (Supplementary Table S1). DNA–DNA relatedness between the two strains was 49.5 %. The results above indicated sufficient genotypic evidence for their assignment to separate novel species (Wayne *et al.*, 1987).

Biochemical data showed that the cell walls of the new isolates contains the L-Orn–D-Asp-based type of peptidoglycan that is also found in other *Halobacillus* species (Spring *et al.*, 1996). MK-7 was detected as the main menaquinone of strains IS-Hb4^T and IS-Hb7^T. Unlike other *Halobacillus* species, which often produce the predominant fatty acids anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{14:0}, iso-C_{15:0} and iso-C_{16:0}, the deep-sea isolates IS-Hb4^T and IS-Hb7^T

showed high percentages of C_{16:0} (28.22 and 20.28 %, respectively), C_{16:1ω7c} alcohol (12.25 and 8.47 %), C_{18:1ω7c} (30.99 and 25.98 %) and C_{19:0} cyc (18.99 and 14.54 %) in their fatty acid profiles. Furthermore, some acids, such as C_{12:0ω7} and C_{17:0} cyc, were found in these isolates for the first time within the halobacilli (Table 1). The DNA G + C contents of strains IS-Hb4^T and IS-Hb7^T were 43.3 and 42.1 mol%, respectively (Table 2).

The deep-sea isolates shared common morphological and physiological characteristics with *Halobacillus* type strains as noted in the species descriptions and Table 2. Under increased hydrostatic pressure, growth was observed in all cases from 0.1 to 30.0 MPa. Weak growth of strain IS-Hb4^T and IS-Hb7^T (small colonies of about 0.5 mm in diameter after 48 h, with slow growth and little increase in OD₆₀₀) was observed under anaerobic conditions and strain IS-Hb4^T (but not strain IS-Hb7^T) reduced nitrate to nitrite; these differences from other species of the genus *Halobacillus* show a tendency of adaptation of these strains to the deep-sea environment. If so, they are the first members of the genus *Halobacillus* to share anaerobic activity with *Bacillus* species (Marino *et al.*, 2000, 2001; Ye *et al.*, 2000).

Description of *Halobacillus profundus* sp. nov.

Halobacillus profundus (pro.fun'di. L. gen. n. *profundus* of the depths of the sea, of the deep-sea).

Rod-shaped, Gram-positive, non-motile cells, 0.5–1.0 by 1.0–2.9 µm, occur singly or in pairs or short chains (Fig. 2a). Endospores are spherical or ellipsoidal and are located in a central position. Colonies are circular, convex with entire margins, pale yellow in colour and 1.5–2.5 mm in diameter after 3 days. Temperature range for growth is 9–47 °C (optimum 25 °C). Growth occurs in medium

Fig. 1. Neighbour-joining phylogenetic tree showing relationships of the deep-sea strains IS-Hb4^T and IS-Hb7^T to other type strains in genus *Halobacillus* and other related bacteria based on 16S rRNA gene sequences. Values of bootstrap greater than or equal to 50 % (from 1000 replications) are shown at nodes. Bar, 0.02 substitutions per site.

Table 1. Cellular fatty acid composition of strains IS-Hb4^T and IS-Hb7^T and type strains of the genus *Halobacillus*

Strains: 1, strain IS-Hb4^T; 2, strain IS-Hb7^T; 3, *H. halophilus* 3^T (data from Claus *et al.*, 1983); 4, *H. trueperi* SL-5^T (Spring *et al.*, 1996); 5, *H. litoralis* SL-4^T (Spring *et al.*, 1996); 6, *H. karajensis* MA-2^T (Amoozegar *et al.*, 2003b); 7, *H. salinus* HSL-3^T (Yoon *et al.*, 2003); 8, *H. locisalis* MSS-155^T (Yoon *et al.*, 2004); 9, *H. yeomjeoni* MSS-402^T (Yoon *et al.*, 2005); 10, *H. aidingensis* AD-6^T (Liu *et al.*, 2005); 11, *H. dabanensis* D-8^T (Liu *et al.*, 2005). Strains IS-Hb4^T and IS-Hb7^T were cultured at their optimal temperature (25 °C) and NaCl concentration (10 and 6 % NaCl, respectively). Values are percentages of fatty acids. ND, Not detected/not reported.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
C _{14:0}	0.38	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C _{15:0}	ND	ND	1.5	ND	1.1	0.3	1.6	1.1	ND	0.26	2.93
C _{16:0}	28.22	20.28	0.9	0.9	0.6	0.1	1.0	0.9	1.6	2.28	2.03
C _{18:0}	2.18	3.05	ND	ND	ND	ND	ND	ND	ND	ND	ND
anteiso-C _{13:0}	ND	ND	0.4	ND	0.4	ND	ND	0.5	ND	0.08	0.07
anteiso-C _{15:0}	ND	11.07	47.3	25.3	57.0	42.4	31.7	42.0	40.4	42.04	49.7
anteiso-C _{17:0}	ND	3.72	11.9	6.5	8.2	16.0	6.2	13.0	23.0	15.68	18.63
iso-C _{14:0}	ND	ND	12.2	21.7	6.4	2.0	9.4	11.2	3.4	2.34	1.10
iso-C _{15:0}	ND	0.72	7.5	7.7	15.8	11.3	26.3	8.4	8.8	13.47	7.45
iso-C _{16:0}	ND	0.73	15.2	31.5	5.4	6.9	15.7	15.9	19.3	7.47	8.15
iso-C _{17:0}	ND	ND	1.2	2.1	1.5	5.0	4.2	1.4	3.5	4.36	2.22
C _{12:0} ω7*	0.53	8.63	ND	ND	ND	ND	ND	ND	ND	ND	ND
C _{12:1} ω1c	0.16	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C _{16:1} ω7c alcohol	12.25	8.47	1.8	3.8	2.0	9.7	4.0	5.7	ND	3.01	4.11
C _{16:1} ω11c	ND	ND	ND	ND	ND	0.9	ND	ND	ND	0.67	0.66
iso-C _{17:1} ω10c	ND	ND	ND	ND	0.2	1.1	ND	ND	ND	1.42	0.35
C _{18:1} ω7c	30.99	25.98	ND	ND	ND	ND	ND	ND	ND	ND	ND
C _{17:0} cyc	6.30	2.81	ND	ND	ND	ND	ND	ND	ND	ND	ND
C _{19:0} cyc	18.99	14.54	ND	ND	ND	ND	ND	ND	ND	ND	ND

*Unknown fatty acid.

containing 0.5–30 % (w/v) NaCl (optimum 10 %). The pH range for growth is 5.5–10.0 (optimum pH 7.5–8.0). Weak growth is observed under anaerobic conditions. Nitrate is variably reduced to nitrite. Catalase- and oxidase-positive. Acid-fastness, phenylalanine deaminase and urease tests are negative. Tweens 20, 40 and 80, starch, casein and aesculin are hydrolysed. Gelatin, dextrin, α-cyclodextrin and glycogen are not hydrolysed. Acid is produced from D-fructose, maltose, D-glucose, D-xylose, D-mannitol, maltose, mannose, rhamnose, sucrose, trehalose, raffinose, mannitol, sorbitol and N-acetylglucosamine. Acid is not produced from D-tagatose, cellobiose, D-galactose, melibiose, cellobiose or inositol. The following substrates are utilized as single sources of carbon: N-acetyl-D-glucosamine, i-erythritol, α-D-lactose, D-mannitol, pyruvic acid methyl ester, α-cyclodextrin, Tweens 40 and 80, α-ketoglutaric acid, α-ketobutyric acid, α-ketovaleric acid, D-saccharic acid, sebacic acid, L-glutamic acid, glycyl L-aspartic acid and D-serine (Supplementary Table S2). The cell wall contains peptidoglycan based on L-Orn-D-Asp. The major menaquinone is MK-7. Major fatty acids are C_{16:0}, C_{16:1}ω7c alcohol, C_{18:1}ω7c and C_{19:0} cyc. The DNA G + C content of the type strain is 43.3 mol% (determined by CZE).

The type strain, IS-Hb4^T (= JCM 14154^T = DSM 18394^T), was isolated from a carbonate rock collected from a 642

m-deep methane cold seep in Kuroshima Knoll, south-western Japan.

Description of *Halobacillus kuroshimensis* sp. nov.

Halobacillus kuroshimensis (ku.ro.shi.men'sis. N.L. masc. adj. *kuroshimensis* from Kuroshima, Japan, where the type strain was isolated).

Rod-shaped, Gram-positive, non-motile cells, 0.5–1.0 by 1.6–3.3 µm, occur singly or in pairs or short chains (Fig. 2b). Endospores are spherical or ellipsoidal and are located in a terminal position. Colonies are circular, convex with entire margins, yellow–orange in colour and 1.5–2.5 mm in diameter after 3 days. Temperature range for growth is 9–48 °C (optimum 25 °C). Growth occurs in medium containing 0.5–25 % (w/v) NaCl (optimum 6 %). pH range for growth is 5.5–10.0 (optimum pH 7.5–8.0). Growth occurs weakly under anaerobic conditions. Nitrate is not reduced to nitrite. Catalase- and oxidase-positive. Acid-fastness, phenylalanine deaminase and urease tests are negative. Tweens 20, 40 and 80, starch, gelatin, casein and aesculin are hydrolysed. Dextrin, α-cyclodextrin and glycogen are not hydrolysed. Acid is produced from D-fructose, D-glucose, maltose, mannose, inositol, glycerol,

Table 2. Phenotypic features of strains IS-Hb4^T and IS-Hb7^T in comparison with other *Halobacillus* species

Species/strains: 1, strain IS-Hb4^T; 2, strain IS-Hb7^T; 3, *H. halophilus* (data from Claus *et al.*, 1983); 4, *H. trueperi* (Spring *et al.*, 1996); 5, *H. litoralis* (Spring *et al.*, 1996); 6, *H. karajensis* (Amoozegar *et al.*, 2003b); 7, *H. salinus* (Yoon *et al.*, 2003); 8, *H. locialis* (Yoon *et al.*, 2004); 9, *H. yeomjeoni* (Yoon *et al.*, 2005); 10, *H. aidingensis* (Liu *et al.*, 2005); 11, *H. dabanensis* (Liu *et al.*, 2005). All strains are positive in Gram staining, catalase and oxidase and negative for urease activity. +, Positive; –, negative; W, weak; V, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Cell morphology	Rods	Rods	Coccoid	Rods	Rods	Rods	Rods	Rods	Rods	Clubs	Rods
Mobility	–	–	+	+	+	–	+	+	+	+	+
Spore morphology*											
Shape	E/S	E/S	S	E/S	E	E/S	E/S	E	E	E	E
Position	C	T	C/L	C/ST	C/ST	C/ST	C/ST	C/ST	C/ST	C/ST	C/ST
Growth conditions											
Temperature (°C)	9–47	9–48	15–40	10–44	10–43	10–49	10–45	10–42	15–48	15–40	15–50
pH	5.5–10.0	5.5–10.0	7.0–9.0	6.0–9.5	6.0–9.5	6.0–9.6	ND	5.0–9.5	6.0–ND	6.0–10.0	5.0–11.0
NaCl concentration (% w/v)	0.5–30.0	0.5–25.0	2.0–20.0	0.5–30.0	0.5–25.0	1.0–24.0	0.0–23.0	>0–23.0	0.5–21.0	0.5–20.0	0.5–25.0
Nitrate reduction	V	–	–	–	–	–	–	–	–	–	–
Hydrolysis of:											
Gelatin	–	+	+	+	+	+	+	–	+	+	–
Aesculin	+	+	–	–	–	+	+	+	–	–	–
Casein	+	+	+	–	–	+	+	–	+	+	+
Starch	+	+	–	–	–	+	–	+	–	+	+
Tween 80	+	+	+	–	–	–	+	+	+	–	–
Acid production from:											
D-Galactose	–	–	–	+	–	–	W	–	–	–	–
Sucrose	+	W	–	+	+	–	+	+	+	+	+
D-Xylose	+	–	–	–	+	–	–	–	–	–	+
D-Mannitol	+	–	–	–	+	+	+	–	+	+	+
D-Trehalose	+	–	–	+	+	ND	+	+	+	+	+
Maltose	+	+	–	+	+	+	+	–	+	+	+
DNA G+C content (mol%)	43.3	42.1	40.1–40.9	43.0	42.0	41.3	45.0	44.0	42.9	42.2	41.4

*Spore shape: E, ellipsoidal; S, spherical. Spore position: C, central; L, lateral; ST, subterminal; T, terminal.

Fig. 2. Phase-contrast micrographs of cells of strains IS-Hb4^T (a) and IS-Hb7^T (b). Bars, 5 µm.

N-acetylglucosamine, sucrose and cellobiose. Acid is not produced from D-tagatose, D-galactose, salicin, D-xylose, D-mannitol, D-trehalose or melibiose. D-Xylose, α -D-glucose, *myo*-inositol, maltose, D-mannose and Tweens 40 and 80 are utilized as single carbon sources (Supplementary Table S2). The cell wall contains peptidoglycan based on L-Orn-D-Asp. The major menaquinone is MK-7. Fatty acids C_{16:1 ω 7c} alcohol, C_{18:1 ω 7c} and C_{19:0} cyc are abundant. The DNA G+C content of the type strain is 42.1 mol% (determined by CZE).

The type strain, IS-Hb7^T (=JCM 14155^T=DSM 18393^T), was isolated from the same sample as strain IS-Hb4^T.

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