

Anaerosporobacter mobilis gen. nov., sp. nov., isolated from forest soil

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A strictly anaerobic, Gram-positive, endospore-forming bacterium, strain HY-37-4^T, was isolated from a forest-soil sample collected in Jeju, Republic of Korea. The cells were motile rods with peritrichous flagella. Strain HY-37-4^T fermented various carbohydrates and the end products from glucose were formate, acetate and H₂. The major cellular fatty acids were C_{16:0}, C_{16:0} 3-OH and iso-C_{17:1} / anteiso B. The G + C content of the DNA was 41 mol%. A phylogenetic analysis based on 16S rRNA sequence data indicated that the forest isolate was most closely related to *Clostridium herbivorans*, *Clostridium populeti*, *Clostridium polysaccharolyticum* and *Eubacterium xylanophilum*, which belong to *Clostridium* cluster XIVa. However, the low levels of 16S rRNA gene sequence similarity (92.3–93.9 %) with respect to these taxa indicate that strain HY-37-4^T represents a novel species. Several phenotypic characteristics readily allowed the isolate to be distinguished from other phylogenetically related taxa. On the basis of the polyphasic evidence, strain HY-37-4^T represents a novel taxon, for which the name *Anaerosporobacter mobilis* gen. nov., sp. nov. is proposed. The type strain is HY-37-4^T (=IMSNU 40011^T=KCTC 5027^T=DSM 15930^T).

During the course of a study on the diversity of culturable, mesophilic, strictly anaerobic bacteria from natural environments in Korea, a bacterial strain, designated HY-37-4^T, was isolated from a forest-soil sample collected in Jeju, Republic of Korea. In this study, we report the taxonomic characterization of this forest isolate, which is considered to be a member of the family *Clostridiaceae*. On the basis of the polyphasic evidence, it is proposed that this isolate be classified as a novel taxon.

Strain HY-37-4^T was isolated by using the standard dilution plating method. The organism was cultivated on a Reinforced Clostridial (RC; Difco) agar plate at 30 °C under anaerobic conditions (N₂/CO₂/H₂; 90:5:5, by vol.) and maintained as glycerol suspensions (20 %, w/v) at –80 °C.

Bacterial DNA preparation, and PCR amplification and sequencing of 16S rRNA gene were carried out as described previously (Chun & Goodfellow, 1995). The sequence of the isolate was aligned manually against sequences of clostridial species obtained from the GenBank database.

Phylogenetic analysis was performed by using the neighbour-joining method (Saitou & Nei, 1987). Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant tree topology was evaluated in bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at <http://chunlab.snu.ac.kr/jphydit/>) and PAUP, version 4.0 (Swofford, 1998), as described previously (Yi & Chun, 2006).

An almost-complete 16S rRNA gene sequence (1390 bp) of the isolate was obtained and used for the initial BLAST search against the GenBank database. On the basis of 16S rRNA gene sequence similarity, the closest bacterial relatives were *Clostridium herbivorans* (93.9 %), *Clostridium populeti* (93.3 %), *Clostridium polysaccharolyticum* (92.6 %) and *Eubacterium xylanophilum* (92.3 %); all belong to *Clostridium* cluster XIVa, defined by Collins *et al.* (1994). A phylogenetic tree (Fig. 1) based on the neighbour-joining method not only confirmed that our isolate is a member of cluster XIVa but also showed that it represents a distinct phyletic line; this conclusion was also supported by bootstrap analysis and the remaining two tree-making algorithms. It is evident that our isolate is not associated with

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HY-37-4^T is AY534872.

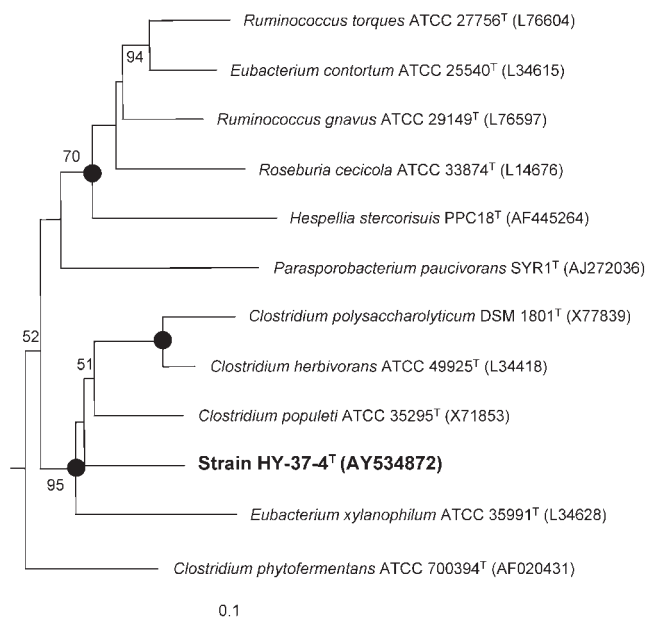


Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, for strain HY-37-4^T and closely related taxa within cluster XIVA of the *Clostridium* subphylum of low-G+C, Gram-positive bacteria. Numbers at the nodes indicate percentages of 1000 bootstrap resamplings; only values >50% are shown. Filled circles indicate that the corresponding nodes (groupings) were recovered using more than three tree-infering methods. *Clostridium perfringens* ATCC 13124^T (M59103) was used as an outgroup (not shown). Bar, 0.1 nt substitutions per position.

the type species of the genus *Clostridium*, *Clostridium butyricum*.

The 16S rRNA gene-based phylogenetic study of Collins *et al.* (1994) and subsequent studies (Rainey & Stackebrandt, 1993; Stackebrandt *et al.*, 1999) demonstrated that the known members of the genus *Clostridium* are polyphetic, and more than half of the species are actually not closely related to the type species, *C. butyricum*; the problem arises mainly from the lack of taxonomic characteristics suitable for describing a novel genus comprising endospore-forming, strictly anaerobic bacteria. On the basis of the low levels of 16S rRNA gene sequence similarity with respect to related taxa and the topologies of the phylogenetic trees, it is reasonable to conclude that strain HY-37-4^T represents a novel genus rather than a novel species of the genus *Clostridium*.

Gram-staining and KOH tests were carried out according to Johnson *et al.* (1995) and Powers (1995), respectively. Cell morphology was observed with cells grown on an RC agar plate at 30 °C for 72 h, using light and scanning electron microscopy. Endospores were stained negatively by using the method of Cappuccino & Sherman (1992) and were observed using light microscopy. Motility was checked in a Hungate tube (Bellco) containing RC semi-solid

medium and motility semi-solid medium (containing, 1 distilled water⁻¹, 5.0 g tryptose, 5.0 g NaCl and 5.0 g agar). The flagellar type was determined by transmission electron microscopy of cells stained negatively with 2% (w/v) uranyl acetate. Hydrogen peroxide (3%, v/v) was used to test for catalase activity. Strain HY-37-4^T was tested to determine growth ranges with respect to temperature (between 5 and 50 °C; increments of 5 °C), pH (between pH 5.0 and 9.5; increments of 0.5 pH units) and NaCl concentration (between 0 and 4.5%, w/v; increments of 0.5%); basal medium with 1% (w/v) glucose, as described by Mechichi *et al.* (1999), was used.

Substrate-utilization tests were carried out at 30 °C and an initial pH of 7.0, using the basal medium of Mechichi *et al.* (1999), MI medium (Warnick *et al.*, 2002) and the commercially available API 20A system (bioMérieux). The substrates tested were L-arabinose, D-cellobiose, cellulose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-raffinose, starch, sucrose, xylan, D-xylose, glycerol, D-mannitol, rhamnose, D-ribose, D-sorbitol, trehalose, peptone (0.1%, w/v) and yeast extract. All substrates were examined at a final concentration of 0.2%, with the exception of peptone (0.1%, w/v), xylan (1%, w/v) and cellulose (1%, w/v). The utilization of xylan (from larchwood; Sigma) and cellulose (microcrystalline powder; Aldrich) was determined using an agar-plate method with 1.5% agar. The results were recorded after 2 weeks incubation in the basal medium and MI medium, and within 48 h for the API 20A system. The API 20A system was also used to determine indole production and hydrolysis of urea, gelatin and aesculin. The end products of glucose fermentation were analysed using the method described by Jeong *et al.* (2004).

For the determination of the G+C content, DNA was extracted and purified according to the method described previously (Wolff & Gemmill, 1997). The G+C content (mol%) of the DNA was determined using HPLC of the deoxyribonucleosides, as described by Mesbah *et al.* (1989), using a reversed-phase column (Supelcosil LC-18 S; Supelco). Fatty acid methyl ester analysis was performed by GLC according to the instructions of the Microbial Identification System (MIDI).

Strain HY-37-4^T was KOH-negative and stained Gram-positive. No growth occurred under aerobic conditions. Endospore formation was observed at the mid-exponential phase of growth in a liquid culture of the basal medium containing glucose. The spores were terminal and were oblong to oval in shape. Cell motility was observed, and transmission electron microscopy revealed the presence of peritrichous flagella. The cells grew at 15–40 °C (optimum, 30 °C), at pH 5.5–9.0 (optimum, pH 6.5–7.0) and at NaCl concentrations of 0–3.0% (w/v) (optimum, 0.5%). The substrate-utilization patterns obtained in tests using basal medium, MI medium and the API 20A system were identical: the details are presented in the genus and species descriptions. With 20 mM glucose as the sole carbon

source, the isolate produced formate (1.21 mmol l⁻¹), acetate (2.42 mmol l⁻¹) and hydrogen (5.44 mmol l⁻¹) as the major fermentation products; with 20 mM glucose supplemented with 0.1 % yeast extract, it produced formate (5.55 mmol l⁻¹), acetate (4.07 mmol l⁻¹) and hydrogen (6.36 mmol l⁻¹). The DNA G + C content of strain HY-37-4^T was 41 mol%. The cellular fatty acid profile consisted of C_{16:0} (24.0 %), C_{16:0} 3-OH (21.8 %), iso-C_{17:1} I/anteiso B (11.9 %), C_{18:1}ω7c (7.8 %), C_{16:1}ω7c and/or iso-C_{15:0} 2-OH (6.5 %), C_{16:1}ω9c (4.1 %), C_{18:1}ω9c (4.0 %), an unknown fatty acid (equivalent chain-length, 14.959) (3.8 %), 10 methyl-C_{19:0} (3.4 %), C_{13:1} AT 12–13 (2.7 %), C_{14:0} 3-OH and/or C_{16:1} iso I (2.7 %), C_{14:0} (1.8 %), and C_{15:1} iso H and/or C_{13:0} 3-OH (1.6 %). Several phenotypic properties that serve to differentiate strain HY-37-4^T from phylogenetically related bacteria have been determined (see Table 1).

On the basis of the polyphasic evidence presented here, strain HY-37-4^T should be classified as representing a novel genus and species, for which we propose the name *Anaerosporebacter mobilis* gen. nov., sp. nov.

Description of *Anaerosporebacter* gen. nov.

Anaerosporebacter (An.a.e.ro.spo.ro.bac'ter. Gr. pref. *an-* without; Gr. n. *aer* air; Gr. n. *spora* a seed, and, in biology, a spore; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Anaerosporebacter* an anaerobic, spore-forming, rod-shaped bacterium).

Gram-positive, endospore-forming, strictly anaerobic, mesophilic and neutrophilic rods. Motile by means of peritrichous flagella. Produces almost oval terminal endospores. Various sugars, including glucose, are fermented. The major end products from glucose are formate, acetate and H₂. Catalase- and urease-negative. Indole is not produced. Aesculin is hydrolysed, but gelatin is not.

The type species is *Anaerosporebacter mobilis*. Phylogenetically, the genus is a member of the XIVa cluster of the low-G + C *Clostridiales* group (Collins *et al.*, 1994).

Description of *Anaerosporebacter mobilis* sp. nov.

Anaerosporebacter mobilis (mo.bi'lis. L. masc. adj., *mobilis* motile).

Cells are 0.5–0.6 µm in width by 2.3–5.5 µm in length. Colonies on RC agar plates are approximately 1.0–2.0 mm in diameter, circular with erose edges, convex, translucent to opaque, greyish-white in colour and slightly smooth after 72 h. Grows optimally at 30 °C and pH 6.5–7.0; the optimal NaCl concentration is 0.5 % (w/v). Utilizes a wide range of saccharides as substrates, including L-arabinose, D-cellobiose, cellulose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-raffinose, salicin, starch, sucrose, xylan and D-xylose, but does not produce acid from glycerol, D-mannitol, melezitose, rhamnose, D-ribose, D-sorbitol, trehalose, peptone (0.1 %, w/v) or yeast extract

Table 1. Characteristics that differentiate strain HY-37-4^T from its nearest phylogenetic relatives

Strains: 1, HY-37-4^T; 2, *Clostridium herbivorans* ATCC 49925^T; 3, *Clostridium phytofermentans* ATCC 700394^T; 4, *Eubacterium xylanophilum* ATCC 35991^T; 5, *Ruminococcus gnavus* ATCC 29149^T; 6, *Roseburia cecicola* ATCC 33874^T. All strains are members of *Clostridium* cluster XIVa as defined by Collins *et al.* (1994). Symbols: +, positive; –, negative; w, weakly positive; ND, not determined. Data from Varel *et al.* (1995), Warnick *et al.* (2002), van Gylswyk & van der Toorn (1985), Moore *et al.* (1976) and Stanton & Savage (1983) and this study.

Characteristic	1	2	3	4	5	6
Gram stain	+	+	–	+	+	–
Cell shape	Rod	Rod	Rod	Coccoid rod	Coccus	Rod
Sporulation	+	+	+	–	–	–
Motility	+	+	+	+	–	+
DNA G + C content (mol%)	41	38	36	39	43	42
Optimum growth temperature (°C)	30	39–42	37	ND	37	37
Aesculin hydrolysis	+	–	–	–	+	–
Utilization of:						
L-Arabinose	+	–	w	–	+	ND
D-Cellobiose	+	+	+	+	–	+
D-Fructose	+	–	w	–	+	–
D-Galactose	+	–	+	–	+	+
D-Glucose	+	–	+	–	+	+
Lactose	+	–	+	–	–	–
Maltose	+	+	+	–	+	+
D-Mannose	+	–	+	–	–	ND
D-Raffinose	+	–	ND	–	+	+
Sucrose	+	–	–	–	+	+
D-Xylose	+	–	+	–	+	+

(0.2 %, w/v). The major cellular fatty acids are C_{16:0}, C_{16:0} 3-OH and iso-C_{17:1} I/anteiso B. The G + C content of the DNA of the type strain is 41 mol%.

The type strain, HY-37-4^T (=IMSNU 40011^T=KCTC 5027^T=DSM 15930^T), was isolated from a forest-soil sample from Jeju, Republic of Korea.

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