Acetoanaerobium pronyense sp. nov., an anaerobic alkaliphilic bacterium isolated from a carbonate chimney of the Prony Hydrothermal Field (New Caledonia)

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A novel anaerobic bacterial strain, ST07-YE^T, was isolated from a carbonate chimney of the Prony Hydrothermal Field (PHF) in New Caledonia. Cells were Gram-stain-positive, straight rods (0.7–0.8 × 3.0–5.0 μm) and motile by means of lateral flagella. Strain ST07-YE^T was mesophilic (optimum 35 °C), moderately alkaliphilic and halotolerant (optimum pH 8.7 and 5 g l^{-1} NaCl). Elemental sulfur, sulfate, thiosulfate, sulfite, nitrate and nitrite were not used as terminal electron acceptors. Yeast extract, peptone, tryptone, Casamino acids, crotonate, pyruvate, galactose, maltose, sucrose, ribose, trehalose and glucose were used as carbon sources. Glucose fermentation led to acetate, H_2 and CO_2 formation. Arginine, serine, histidine, lysine, methionine and cysteine improved growth, but the Stickland reaction was negative for the combinations of amino acids tested. The major metabolic products from yeast extract fermentation were H_2, CO2, acetate, butyrate, isobutyrate, isovalerate and propionate. The predominant cellular fatty acids were $C_{16:0}$, $C_{16:1}$*cis*9, $C_{14:0}$ and $C_{16:1}$*cis*7 (>5 % of total fatty acids). The G + C content of the genomic DNA was 32.9 mol%. Phylogenetic analysis revealed that strain ST07-YE^T was most closely related to *Clostridium sticklandii* DSM 519^T and *Acetoanaerobium noterae* NOT-3^T (96.7 % and 96.8 % 16S rRNA gene sequence similarity, respectively). On the basis of phylogenetic, chemotaxonomic and physiological properties, strain ST07-YE^T is proposed to represent a novel species of the genus *Acetoanaerobium* (order *Clostridiales,* phylum *Firmicutes*) with the name *Acetoanaerobium pronyense* sp. nov. The type strain is ST07-YE^T (=DSM 27512^T=JCM 19400^T).

The shallow marine Prony Hydrothermal Field (PHF) located in Prony Bay, in the south of New Caledonia, comprises submarine edifices with the 'Needle of Prony' being the most famous. This 35 m-high edifice was previously described as a coral formation before its hydrothermal origin was established as confirmed by the emission of highly alkaline fluids of low salinity at the top of the chimney (Launay & Fontes, 1985; Magnier, 1979). Subsequently, several other hydrothermal sites have been discovered at various depths in the bay (up to 50 m below sea level) (Pelletier *et al.*, 2006). These extreme environments are mainly driven by serpentinization reactions leading to high-pH fluids (up to pH 11.2) together with H_2 and CH_4 formation (Launay & Fontes, 1985; Monnin *et al.*, 2014). Serpentinization occurs in numerous places on Earth, in both marine and continental contexts (Schrenk *et al.*, 2013). Lost City Hydrothermal Field (LCHF) located 750 m below sea level, near the Mid-Atlantic Ridge, is one of the most-studied serpentinized hydrothermal systems (Kelley *et al.*, 2001). It hosts carbonate chimneys up to 60 m high and generates fluids with moderate temperatures (40–90 °C at chimney vents) and high pH (9–10.8). Although LCHF was the subject of several microbial diversity surveys

Abbreviations: LCHF, Lost City Hydrothermal Field; PHF, Prony Hydrothermal Field.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ST07-YE^T is KJ174056.

Three supplementary figures and a supplementary table are available with the online Supplementary Material.

(Brazelton *et al.*, 2010; Kelley *et al.*, 2005; Schrenk *et al.*, 2004), the isolation and characterization of micro-organisms originating from this deep-sea alkaline site have not been reported so far. In contrast, some culture-dependent studies have been performed in terrestrial alkaline ecosystems (e.g. the ophiolitic complex of Semail in Oman, Cabeço de Vide Aquifer in Portugal and The Cedars springs in California), which provided evidence of heterotrophic aerobes (e.g. *Actinobacteria*, *Staphylococcus, Bacillales*) and also hydrogenotrophic aerobes (e.g. '*Serpentinomonas*') inhabiting these alkaline ecosystems (Bath *et al.*, 1987; Suzuki *et al.*, 2014; Tiago *et al.*, 2004). At the time of writing, the isolation and cultivation of micro-organisms originating from hydrothermal serpentinized marine ecosystems are restricted to the PHF from where *Vallitalea pronyensis* and '*Alkaliphilus hydrothermalis*' have been isolated and recently characterized (Ben Aissa *et al.*, 2014, 2015).

Molecular analyses based on 16S rRNA and functional genes performed on chimney samples of the Needle of Prony (PHF) collected by scuba diving during the HYDROPRONY oceanographic cruise in 2011 (Pelletier *et al.*, 2011) revealed a diverse microbial community dominated by *Firmicutes, Chloroflexi* and *Proteobacteria* within the *Bacteria* and *Methanosarcinales* within the *Archaea* (Quéméneur *et al.*, 2014). Further cultivation experiments were conducted to look for novel anaerobic micro-organisms originating from this needle. Here we report on the isolation of strain ST07-YE^T representing a novel species within the order *Clostridiales*.

The Needle of Prony, also noted ST07, is located in the north of Prony Bay (22° 19.796′ S 166° 50.058′ E) (Monnin *et al.*, 2014; Pelletier *et al.*, 2011). The top part of the chimney was collected at 16.5 m below sea level by scuba diving in November 2011, during the HYDROPRONY oceanographic cruise. It was cut into transversal sections (about 10 cm thick) (Fig. S1, available in the online Supplementary Material). Each section was subsampled along a radial from the centre to the outside (each about 20 cm wide). The inner part associated with the alkaline hydrothermal fluid circulation was further used. The pH and salinity of the emitted fluid were 10.2 and 6 g l^{-1}, respectively (Monnin *et al.*, 2014; Quéméneur *et al.*, 2014). Subsamples used for cultivation experiments were crushed in a sterile mortar under a flux of N_2 to ensure anaerobic conditions, then transferred into sterile penicillin vials under N_2 gas phase, and stored at 4 °C until use.

An enrichment culture was obtained anaerobically on a basal medium comprising (per litre): 2 g NaCl, 5 g yeast extract, 1 g NH_4Cl, 0.1 g KH_2PO_4, 0.1 g K_2HPO_4, 0.1 g KCl, 0.1 g $MgCl_2$, 3 g Tris Base and 1 ml Widdel trace elements solution (Widdel & Pfennig, 1982). The medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature, dispensed into Hungate tubes (5 ml) and degased under N_2/CO_2 (80 : 20, v/v). After sterilization by autoclaving, 0.1 ml $Na_2S.9H_2O$ (3 %, w/v) was added to 5 ml medium in a Hungate tube. The pH was adjusted to 9.0 using 8 % (w/v) Na_2CO_3. The culture media were inoculated with 0.5 g homogeneous chimney sample and incubated at 30 °C. Isolation of colonies was performed twice consecutively by the roll-tube method on 1.6 % (w/v) agar medium (Hungate, 1969). Colonies obtained after 3 days incubation at 30 °C were creamy yellow, round with smooth edges and a diameter of 0.5–1 mm. Several axenic cultures obtained by repeated serial dilutions in liquid medium exhibited the same phylogenic inference. Among them, strain ST07-YE^T was selected for further characterization.

Isolation of genomic DNA was performed from 5 ml culture, using the Wizard Genomic DNA Purification kit (Promega) following the manufacturer's instructions. In order to amplify the 16S rRNA gene by PCR, a PCR mixture containing (total 50 µl) 0.4 pmol µl^{-1} of each primer [27F and 1492R (Lane, 1991)], 1 µl of genomic DNA template and 1 × reaction buffer (GoTaq Hot Start Green Master Mix; Promega) was used. The PCR was carried out using a T100 Thermal Cycler (Bio-Rad) with the following conditions: 2 min at 95 °C, 30 cycles of 30 s at 94 °C, 30 s at 50 °C and 90 s at 72 °C, followed by 5 min at 72 °C. Sanger sequencing of the PCR product was performed at GATC Biotech AG (Germany).

The 16S rRNA gene sequence obtained was compared with sequences in the GenBank database using the BLASTN search (Altschul *et al.*, 1990). A taxonomic assignment of ST07-YE^T 16S rRNA gene sequence was based on RDP naïve Bayesian rRNA Classifier (current RDP Classifier version 2.6 trained on 16S rRNA training set 9) (Wang *et al.*, 2007). A multiple alignment was built using the MUSCLE program (Edgar, 2004) implemented in the MEGA6 software package (Tamura *et al.*, 2013). All positions containing gaps and missing data were eliminated. Phylogenetic trees were reconstructed in the MEGA6 software package using the neighbour-joining method (Maximum Composite Likelihood as substitution model) with bootstrap values of 1000 or 1500 (Felsenstein, 1985; Saitou & Nei, 1987) and confirmed by the maximum-likelihood method.

The 16S rRNA gene sequence (1380 bp) analysis (RDP classifier) of strain ST07-YE^T revealed its assignment to the family *Peptostreptococcaceae*, order *Clostridiales*, phylum *Firmicutes* (Fig. S2). According to a BLASTN analysis, strain ST07-YE^T was closely related to environmental sequences originating from diverse environments including oil reservoirs, sediment surface rocks, and activated sludge at pH 10.0 (99.4–99.9 % 16S rRNA gene sequence similarity). The closest phylogenetic cultivated relatives to strain ST07-YE^T were *Acetoanaerobium noterae* NOT-3^T (96.8 %) (Sleat *et al.*, 1985) and *Clostridium sticklandii* DSM 519^T (96.7 %) (Stadtman & McClung, 1957), which were isolated from an oil-drilling site and from soil, respectively (Fig. 1).

The G+C content of genomic DNA of strain ST07 YE^T was determined by the Deutsche Sammlung von

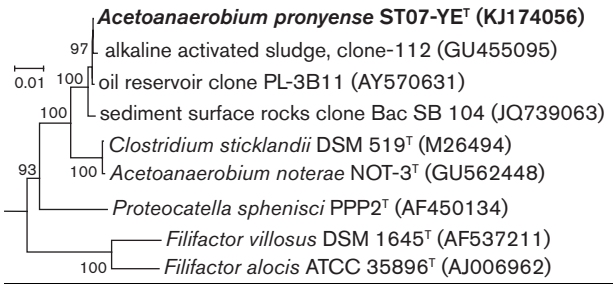

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the position of strain ST07-YE^T among the order *Clostridiales*. A total of 1255 sites were used for the phylogenetic analysis. GenBank accession numbers are indicated in parentheses. The topology corresponds to an unrooted tree obtained by the neighbour-joining method. Numbers at nodes indicate bootstrap values from 1000 replications. Bar, 0.01 substitutions per nucleotide position.

Mikroorganismen und Zellkulturen (DSMZ; Braunschweig, Germany). DNA was isolated and purified by chromatography on hydroxyapatite (Cashion *et al.*, 1977) and the G+C content was determined by using HPLC (Mesbah *et al.*, 1989). The G+C content of the genomic DNA of strain ST07-YE^T was 32.9 mol% (Table 1).

The determination of cellular fatty acids composition was performed by the Identification Service of the DSMZ after extraction using a modified version of the method of Miller (Kuykendall *et al.*, 1988; Miller, 1982). Fatty acids were separated using the Sherlock Microbial Identification system (version 4.0, MIDI) (Sasser, 1990). The major cellular fatty acids of strain ST07-YE^T were $C_{16:0}$ (46.4 % of the total), $C_{16:1}$*cis*9 (14.1 %), $C_{14:0}$ (6.1 %) and $C_{16:1}$*cis*7 (5.5 %) (Table S1).

Antibiotic resistance of strain ST07-YE^T was tested by adding the following antibiotics to the medium: ampicillin, kanamycin, gentamicin, vancomycin, tetracycline, rifampicin and chloramphenicol at 50, 150 or 300 μg ml^{-1} final concentration. The strain was sensitive to all of these antibiotics at 50 μg ml^{-1} final concentration.

The morphology of strain ST07-YE^T was examined using a phase-contrast microscope (ECLIPSE E600; Nikon). Cells were rod-shaped (0.7–0.8 μm in diameter and 3.0–5.0 μm in length) during the exponential growth phase (Fig. 2a). The presence of terminal swollen sporangium with oval shape was observed by phase-contrast microscopy during the stationary growth phase (Fig. 2b). Cells reached up to 13 μm in length, and were motile. Exponentially grown cells were negatively stained with 1 % sodium phosphotungstate (pH 7.0) for flagella observation using a Zeiss EM912 transmission electron microscope. Several lateral flagella

Table 1. Differential characteristics between strain ST07-YE^T, *Acetoanaerobium noterae* NOT-3^T and *Clostridium sticklandii* DSM 519^T

Strains: 1, ST07-YE^T (data from this study); 2, *Acetoanaerobium noterae* NOT-3^T (Sleat *et al.*, 1985); 3, *Clostridium sticklandii* DSM 519^T (this study unless otherwise indicated). ND, Not determined; +, positive; −, negative.

Characteristic	1	2	3
Source	Hydrothermal alkaline chimney, Prony Bay	Notera oil drilling site, Israël	San Francisco Bay black mud*†
Gram reaction/stain	+	−	+
Cell size (μm)	0.7–0.8 × 3.0–5.0	0.8 × 1.0–5.0	0.3–0.5 × 1.3–3.8*
Range (optimum) for growth			
Temperature (°C)	15–40 (35)	(37)	25–45 (30–37)*
Salinity (g l^{-1} NaCl)	0–70 (5)	ND	0–70 (5)
pH	7.2–10.0 (8.7)	6.6–8.4 (7.6)	5.0–8.5 (6.9)
Substrate consumption			
Pyruvate	+	−	+
D-Ribose	+	−	+
Sucrose	+	−	+
Trehalose	+	−	−
D-Galactose	+	−	−†
H_2/CO_2	−	+	−
Stickland reaction	−	ND	+*
DNA G+C content (mol%)	32.9	36.8	31.0*

*Data from Stadtman & McClung (1957).
†Differed from Stadtman & McClung (1957).

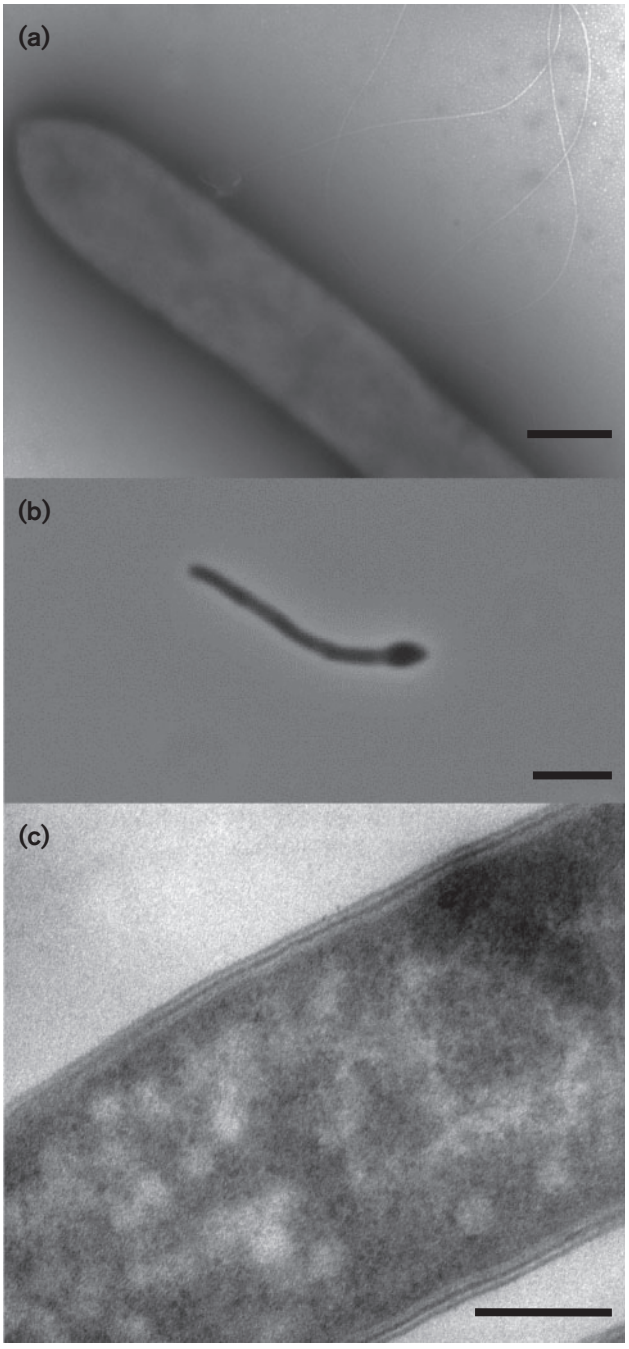

Fig. 2. (a) Transmission electron micrograph of cells of strain ST07-YE^T with several lateral flagella. **(b)** Phase-contrast micrograph of cell of strain ST07-YE^T with a terminal sporangium formation. **(c)** Thin-section electron micrograph of strain ST07-YE^T showing a multi-layered cell wall in the absence of an outer membrane, typical of Gram-positive bacteria. Bars, 0.5 μm (a), 3 μm (b) and 200 nm (c).

were observed (Fig. 2a). For observation of cell wall, cells were fixed with glutaraldehyde, stained with osmium tetraoxyde, and then embedded in EMbed-812. Embedded specimens were sliced into ultrathin sections (90 nm), placed on a grid and stained with uranyl acetate and lead. The cell-wall structure observed by transmission electron microscopy revealed a Gram-positive structure with the presence of a multi-layered cell wall in the absence of an outer membrane, which was confirmed by the Gram-staining of cell membranes (Fig. 2c).

Growth was determined by measuring the optical density at 600 nm (Cary 50 UV-Vis; Varian). Experiments were performed in duplicate. All tested substrates were added to the basal medium in which yeast extract concentration was lowered to 2 g l^{-1}. D-Glucose, lactose, D-mannose, maltose, sucrose, D-ribose, D-xylose, cellobiose, trehalose, D-arabinose, D-fructose, D-galactose, starch, pectin, chitin, acetate, fumarate, lactate, pyruvate, crotonate, oxalate and tartrate were tested at a final concentration of 20 mM, whereas proteinaceous compounds (peptone, tryptone and Casamino acids) were tested at 5 g l^{-1}. L-Amino acids (serine, threonine, glycine, cysteine, alanine, glutamate, valine, isoleucine, proline, methionine, aspartate, leucine, phenylalanine, histidine, asparagine, glutamine, arginine, lysine, histidine and tyrosine) were tested at 10 mM each. Growth on H_2/CO_2 (80 : 20 v/v, 2 bars) was tested in the presence of either Balch vitamins (Balch *et al.*, 1979), 2 mM acetate (carbon source) or 0.2 g l^{-1} yeast extract. Elemental sulfur (1 %, w/v), sulfate (20 mM), thiosulfate (20 mM), sulfite (2 mM), nitrate (20 mM) and nitrite (2 mM) were tested as terminal electron acceptors. End products of metabolism were determined by HPLC and gas phase chromatography after 10 days incubation (Fardeau *et al.*, 2000). H_2S production was determined photometrically at 480 nm as colloidal CuS (Cord-Ruwisch, 1985).

Strain ST07-YE^T grew only under strictly anaerobic conditions within a temperature range of 15 to 40 °C with optimal growth observed at 35 °C (Fig. S3). Growth occurred between pH 7.2 and 10 and the optimum pH for growth was 8.7. The strain did not require NaCl for growth. The optimal NaCl concentration was 5 g l^{-1}; above 10 g l^{-1}, growth decreased sharply and stopped at 80 g l^{-1}. Under optimal conditions thereby determined, the maximal growth rate of strain ST07-YE^T grown on the basal medium containing 5 g l^{-1} yeast extract was 0.14 h^{-1}. Using ST07-YE^T culture media, we determined the optimal salinity and pH conditions for growth of *C. sticklandii* DSM 519^T for comparison purposes. The optimal NaCl concentration for growth of *C. sticklandii* DSM 519^T was 5 g l^{-1}, with growth occurring between 0 and 70 g l^{-1} NaCl. *C. sticklandii* DSM 519^T grew from pH 5.0 to 8.5 with optimal growth at pH 6.9 (Table 1).

Strain ST07-YE^T grew only chemoorganoheterotrophically and used sugars, organic acids, proteinaceous compounds and amino acids. Among tested sugars, D-glucose, maltose, sucrose, D-ribose, trehalose and D-galactose were fermented into acetate H_2, and CO_2. Pyruvate and crotonate were the only organic acids consumed. Pyruvate was degraded to acetate and formate, while crotonate was

disproportionated to acetate and butyrate. The following substrates were not used: lactose, D-mannose, D-xylose, cellobiose, D-arabinose, D-fructose, starch, pectin, chitin, acetate, fumarate, lactate, oxalate and tartrate. Yeast extract, peptone, tryptone and Casamino acids were also fermented. H_2, CO_2, acetate, propionate, butyrate, isobutyrate and isovalerate were the end products of yeast extract metabolism. The addition of arginine, serine, histidine, lysine, methionine and cysteine (at 10 mM final) as single amino acid to the basal culture medium improved growth of strain ST07-YE^T. These amino acids were mainly oxidized to acetate. Besides acetate, butyrate and propionate were found as end products of lysine and methionine fermentation, respectively. The following combinations of amino acids in the basal medium containing 2 g l^{-1} yeast extract were used for testing the ability of strain ST07-YE^T to perform the Stickland reaction: alanine + glycine, isoleucine + methionine, proline + serine, arginine + cysteine, arginine + serine and serine + cysteine. None of the amino acid combinations supported growth. Elemental sulfur, sulfate, thiosulfate, sulfite, nitrate and nitrite were not used as terminal electron acceptors. For comparison purposes, growth of *C. sticklandii* DSM 519^T was tested on proteinaceous compounds and sugars in the same culture conditions as strain ST07-YE^T. *C. sticklandii* DSM 519^T used yeast extract, peptone, tryptone, Casamino acids, D-glucose, maltose, sucrose, D-ribose, crotonate and pyruvate, but did not use lactose, D-mannose, D-xylose, cellobiose, trehalose, D-arabinose, D-fructose, D-galactose, starch, pectin, chitin, acetate, fumarate, lactate, oxalate or tartrate. Differences in the range of substrates used by both bacteria together with *Acetoanaerobium noterae* NOT-3^T are reported in Table 1. One noticeable metabolic feature of *Acetoanaerobium noterae* NOT-3^T is that in contrast to *C. sticklandii* DSM 519^T and strain ST07-YE^T, the former may oxidize hydrogen and reduce CO_2 to acetate (Sleat *et al.*, 1985).

Strain ST07-YE^T, *C. sticklandii* DSM 519^T and *Acetoanaerobium noterae* NOT-3^T may also be distinguished by their genomic DNA G + C content and their optimal pH and pH ranges for growth (Table 1). While *C. sticklandii* DSM 519^T and *Acetoanaerobium noterae* NOT-3^T grew optimally at neutral pH, strain ST07-YE^T should be considered as an anaerobic alkaliphile (optimal pH 8.7) (Table 1).

Interestingly, the alkaliphilic and mesophilic features of strain ST07-YE^T, together with its optimal growth at low salinity fit with the physico-chemical conditions existing inside the PHF chimneys. Therefore, given the broad range of substrates used by strain ST07-YE^T, we may expect this bacterium with possibly other *Firmicutes* retrieved from these alkaline environments to play *in situ* a significant ecological role in organic matter degradation and/or hydrogen production. Indeed, the importance of *Firmicutes*, and especially *Clostridia*, has been underlined in various terrestrial or marine serpentinite-hosted ecosystems where they were shown to be major components of the indigenous microbial communities (Brazelton *et al.*, 2006; Kelley *et al.*, 2005; Quéméneur *et al.*, 2014; Suzuki *et al.*, 2013; Tiago & Veríssimo, 2013). However, it is only recently that anaerobic *Firmicutes* colonizing alkaline marine ecosystems have been isolated and characterized. They include *Vallitalea pronyensis* and '*Alkaliphilus hydrothermalis*', which originated from PHF chimneys (Ben Aissa *et al.*, 2014, 2015). Based on phylogenetic, genetic phenotypic, and chemotaxonomic characteristics, we propose strain ST07-YE^T, also isolated from PHF, should be assigned as a novel species of the family *Peptostreptococcaceae*, order *Clostridiales*. Given that *Acetoanaerobium noterae* NOT-3^T is the closest relative of strain ST07-YE^T and according to the generally accepted classification described by Collins *et al.* (1994) and reported in *Bergey's Manual of Systematic Bacteriology* (Rainey *et al.*, 2009) recommending that 'true' *Clostridium* should be restricted to the rRNA Cluster I, we propose the name *Acetoanaerobium pronyense* sp. nov. for the novel species.

Description of *Acetoanaerobium pronyense* sp. nov.

Acetoanaerobium pronyense (pro.ny.en′se. N.L. neut. adj. *pronyense* of or belonging to Prony underwater hydrothermal field in New Caledonia).

Cells are Gram-stain-positive, straight rods (0.7–0.8 × 3.0–5.0 μm), and motile by means of peritrichous flagella. Terminal swollen sporangium formation with oval shape is observed. Colonies obtained after 3 days incubation at 30 °C are creamy yellow, round with smooth edges and a diameter of 0.5–1.0 mm. Optimal growth conditions are 35 °C, pH 8.7 and 5 g l^{-1} NaCl. Has a fermentative metabolism. Yeast extract, peptone, tryptone, Casamino acids, D-glucose, maltose, sucrose, D-ribose, trehalose, D-galactose, crotonate and pyruvate are used as substrates for growth. Cells produce acetate, H_2 and CO_2 from sugar fermentation, while H_2, CO_2, acetate, butyrate isobutyrate, isovalerate and propionate are end products of yeast extract fermentation. Crotonate is disproportionated to acetate and butyrate. The following substrates are not used: lactose, D-mannose, D-xylose, cellobiose, D-arabinose, D-fructose, starch, pectin, chitin, acetate, fumarate, lactate, oxalate and tartrate. Among amino acids, arginine, serine, histidine, lysine, methionine and cysteine improved growth. The Stickland reaction was negative for all amino acid combinations tested (alanine + glycine, isoleucine + methionine, proline + serine, arginine + cystein, arginine + serine and serine + cystein). Elemental sulfur, sulfate, thiosulfate, sulfite, nitrate and nitrite are not used as terminal electron acceptors. Sensitive to ampicillin, kanamycin, gentamicin, vancomycin, tetracycline, rifampicin and chloramphenicol at 50 μg ml^{-1}. The predominant cellular fatty acids are $C_{16:0}$, $C_{16:1}$*cis*9, $C_{14:0}$ and $C_{16:1}$*cis*7 (> 5% of total fatty acids).

The type strain, ST07-YE^T (=DSM 27512^T=JCM 19400^T), was isolated from a carbonaceous chimney of the alkaline

Prony Hydrothermal Field (PHF) in New Caledonia. The DNA G+C content of the type strain is 32.9 mol%.

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References

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.

Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.

Bath, A. H., Christofi, N., Neal, C., Philip, J. C., Cave, M. R., McKinley, I. G. & Berner, U. (1987). *Trace element and microbiological studies of alkaline groundwaters in Oman, Arabian Gulf: a natural analogue for cement pore-waters.* Report of the Fluid Processes Research Group Report FLPU 87–2 British Geological Survey United Kingdom: Keyworth.

Ben Aissa, F., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Ollivier, B. & Fardeau, M.-L. (2014). *Vallitalea pronyensis* sp. nov., isolated from a marine alkaline hydrothermal chimney. *Int J Syst Evol Microbiol* **64**, 1160–1165.

Ben Aissa, F., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Fardeau, M.-L. & Ollivier, B. (2015). Characterization of *Alkaliphilus hydrothermalis* sp. nov., a novel alkaliphilic anaerobic bacterium, isolated from a carbonaceous chimney of the Prony hydrothermal field, New Caledonia. *Extremophiles* **19**, 183–188.

Brazelton, W. J., Schrenk, M. O., Kelley, D. S. & Baross, J. A. (2006). Methane- and sulfur-metabolizing microbial communities dominate the Lost City hydrothermal field ecosystem. *Appl Environ Microbiol* **72**, 6257–6270.

Brazelton, W. J., Ludwig, K. A., Sogin, M. L., Andreishcheva, E. N., Kelley, D. S., Shen, C.-C., Edwards, R. L. & Baross, J. A. (2010). *Archaea* and *bacteria* with surprising microdiversity show shifts in dominance over 1,000-year time scales in hydrothermal chimneys. *Proc Natl Acad Sci U S A* **107**, 1612–1617.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

Collins, M. D., Lawson, P. A., Willems, A., Cordoba, J. J., Fernandez-Garayzabal, J., Garcia, P., Cai, J., Hippe, H. & Farrow, J. A. E. (1994). The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int J Syst Bacteriol* **44**, 812–826.

Cord-Ruwisch, R. (1985). A quick method for determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbiol Methods* **4**, 33–36.

Edgar, R. C. (2004). MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792–1797.

Fardeau, M. L., Magot, M., Patel, B. K., Thomas, P., Garcia, J. L. & Ollivier, B. (2000). *Thermoanaerobacter subterraneus* sp. nov., a novel thermophile isolated from oilfield water. *Int J Syst Evol Microbiol* **50**, 2141–2149.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Hungate, R. E. (1969). A roll tube method for the cultivation of strict anaerobes. *Methods Microbiol* **3B**, 117–132.

Kelley, D. S., Karson, J. A., Blackman, D. K., Früh-Green, G. L., Butterfield, D. A., Lilley, M. D., Olson, E. J., Schrenk, M. O., Roe, K. K. & other authors (2001). An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. *Nature* **412**, 145–149.

Kelley, D. S., Karson, J. A., Früh-Green, G. L., Yoerger, D. R., Shank, T. M., Butterfield, D. A., Hayes, J. M., Schrenk, M. O., Olson, E. J. & other authors (2005). A serpentinite-hosted ecosystem: the Lost City hydrothermal field. *Science* **307**, 1428–1434.

Kuykendall, L. D., Roy, M. A., O'Neill, J. J. & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* **38**, 358–361.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Launay, J. & Fontes, J.-C. (1985). Les sources thermales de Prony (Nouvelle-Calédonie) et leurs précipités chimiques: Exemple de formation de brucite primaire. *Geol Fr* **1985** (1), 83–100.

Magnier, Y. (1979). Une source thermale sous-marine à Prony: le récif de l'aiguille. *Rossiniana* **3**, 16–17.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Miller, L. T. (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *J Clin Microbiol* **16**, 584–586.

Monnin, C., Chavagnac, V., Boulart, C., Ménez, B., Gérard, M., Gérard, E., Pisapia, C., Quéméneur, M., Erauso, G. & other authors (2014). Fluid chemistry of the low temperature hyperalkaline hydrothermal system of Prony bay (New Caledonia). *Biogeosciences* **11**, 5687–5706.

Pelletier, B., Chevillon, C., Menou, J., Butscher, J., Folcher, E., Geoffray, C., Bore, J., Panche, J. & Perrier, J. (2006). Plongées, forage et cartographie Baie du Prony et Banc Gail, lagon Sud de Nouvelle-Calédonie, campagne 2005-NC-PL du N.O. ALIS 13–17 Juin 2005 et cartographie baie du Prony et canal Woodin N.O. ALIS 25–26 September 2005: Nouméa IRD Sept. 2006. *Missions Sci Terre, Géol-Géophys* **70**, 44.

Pelletier, B., Payri, C., Folcher, E., Butscher, J., Bourgeois, B., Erauso, G., Postec, A., Monnin, C., Gérard, M. & other authors (2011). Campagne HYDROPRONY du N.O. ALIS, 28 octobre–13 novembre 2011. Rapport de mission IRD Nouméa.

Quéméneur, M., Bes, M., Postec, A., Mei, N., Hamelin, J., Monnin, C., Chavagnac, V., Payri, C., Pelletier, B. & other authors (2014). Spatial distribution of microbial communities in the shallow submarine alkaline hydrothermal field of the Prony Bay, New Caledonia. *Environ Microbiol Rep* **6**, 665–674.

Rainey, F. A., Hollen, B. J. & Small, A. (2009). Genus I. *Clostridium* Prazmowski 1880, 23^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, pp. 738–828. Edited by P. De Vos, G. M. Garrity, D. Jones, N. R. Krieg, W. Ludwig, F. A. Rainey, K.-H. Schleifer & W. B. Whitman. New York: Springer.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Schrenk, M. O., Kelley, D. S., Bolton, S. A. & Baross, J. A. (2004). Low archaeal diversity linked to subseafloor geochemical processes at the Lost City Hydrothermal Field, Mid-Atlantic Ridge. *Environ Microbiol* **6**, 1086–1095.

Schrenk, M. O., Brazelton, W. J. & Lang, S. Q. (2013). Serpentinization, carbon, and deep life. *Rev Mineral Geochem* **75**, 575–606.

Sleat, R., Mah, R. A. & Robinson, R. (1985). *Acetoanaerobium noterae* gen. nov., sp. nov.: an anaerobic bacterium that forms acetate from H_2 and CO_2. *Int J Syst Bacteriol* **35**, 10–15.

Stadtman, T. C. & McClung, L. S. (1957). *Clostridium sticklandii* nov. spec. *J Bacteriol* **73**, 218–219.

Suzuki, S., Ishii, S., Wu, A., Cheung, A., Tenney, A., Wanger, G., Kuenen, J. G. & Nealson, K. H. (2013). Microbial diversity in The Cedars, an ultrabasic, ultrareducing, and low salinity serpentinizing ecosystem. *Proc Natl Acad Sci U S A* **110**, 15336–15341.

Suzuki, S., Kuenen, J. G., Schipper, K., van der Velde, S., Ishii, S., Wu, A., Sorokin, D. Y., Tenney, A., Meng, X. & other authors (2014). Physiological and genomic features of highly alkaliphilic hydrogen-utilizing *Betaproteobacteria* from a continental serpentinizing site. *Nat Commun* **5**, 3900.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tiago, I. & Veríssimo, A. (2013). Microbial and functional diversity of a subterrestrial high pH groundwater associated to serpentinization. *Environ Microbiol* **15**, 1687–1706.

Tiago, I., Chung, A. P. & Veríssimo, A. (2004). Bacterial diversity in a nonsaline alkaline environment: heterotrophic aerobic populations. *Appl Environ Microbiol* **70**, 7378–7387.

Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl Environ Microbiol* **73**, 5261–5267.

Widdel, F. & Pfennig, N. (1982). Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids II. Incomplete oxidation of propionate by *Desulfobulbus propionicus* gen. nov., sp. nov. *Arch Microbiol* **131**, 360–365.