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Crassaminicella profunda gen. nov., sp nov., an anaerobic marine bacterium isolated from deep-sea sediments

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Abstract :

A novel, anaerobic, chemo-organotrophic bacterium, designated strain Ra1766HT, was isolated from sediments of the Guaymas basin (Gulf of California, Mexico) taken from a depth of 2002 m. Cells were thin, motile, Gram-stain-positive, flexible rods forming terminal endospores. Strain Ra1766H(T) grew at temperatures of 25-45 degrees C (optimum 30 degrees C), pH 6.7-8.1 (optimum 7.5) and in a salinity of 5-60 g l(-1) NaCl (optimum 30 g l(-1)). It was an obligate heterotrophic bacterium fermenting carbohydrates (glucose and mannose) and organic acids (pyruvate and succinate). Casamino acids and amino acids (glutamate, aspartate and glycine) were also fermented. The main end products from glucose fermentation were acetate, butyrate, ethanol, H-2 and CO2. Sulfate, sulfite, thiosulfate, elemental sulfur, fumarate, nitrate, nitrite and Fe(III) were not used as terminal electron acceptors. The predominant cellular fatty acids were C-14 : 0, C-16:1 omega 7, C-16:1 omega 7 DMA and C-16:0. The main polar lipids consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine and phospholipids. The G +C content of the genomic DNA was 33.7 molo/o. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain Ra1766H(T) was affiliated to cluster XI of the order *Clostridia* les, phylum *Firmicutes*. The closest phylogenetic relative of Ra1766H(T) was *Geosporobacter subterraneus* (94.2% 16S rRNA gene sequence similarity). On the basis of phylogenetic inference and phenotypic properties, strain Ra1766H(T) (=DSM 27501(T)=JCM 19377(T)) is proposed to be the type strain of a novel species of a novel genus, named *Crassaminicella profunda*.

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Guaymas basin, Gulf of California, consists in deep, semi-enclosed basins formed by the 5-6 cm/yr separation of Baja California from mainland Mexico. Hydrothermal vent systems in the south rift of the Guaymas basin were discovered after studying conductive heat flow (Williams *et al.,* 1979) and bottom-water helium in the troughs that mark the spreading axes of this basin (Lupton, 1979). These vents systems are located in an area of high sediment loading with a high biologic productivity in the overlying surface waters resulting in deposition of abundant organic-rich sediments with total organic carbon concentrations up to 4 %. Petroleum is actively produced within the basin sediments as the result of magmatic heating of the thick, organic-rich sediment cover (Simoneit, 1985). Thus, this environment is prone to bacterial activity and hydrocarbon degradation or recycling (Pearson *et al.,* 2005). White or yellow microbial biofilms are common features found at sediment-water interfaces (Gundersen *et al.,* 1992). Molecular studies based on 16S rRNA gene sequences and lipid biomarkers showed that Guaymas basin sediments harbour a great diversity of cultured and non cultured *Bacteria*, *Archaea* and *Eukarya* (Teske *et al.,* 2002 ; Dick & Tebo, 2010). Amongst *Bacteria*, the presence of members of *Firmicutes* has been established (Lakhal *et al.,* 2013; Dick *et al.,* 2006). This study presents the taxonomic characterization of a novel bacterium isolated from sediments of the Guaymas basin. The bacterium was found to show phenotypic and phylogenetic traits that led to its assignment to a novel species of a novel genus within the phylum *Firmicutes*, order *Clostridiales*.

Sediments were collected on the 29 of June 2010 from the Guaymas basin, at the Southern Trough site (27°00’44’’N, 111°24’53’’W, at 2002 m depth), during the BIG 2010 cruise (RV *Atalante*) using the submersible *Nautile* (IFREMER, France). Strain Ra1766H^T was isolated from white mats on the sediments surface at an *in situ* temperature of 25 °C (Lakhal *et al.,* 2013). Sediment sampling was performed with a push core using the port manipulator of the

submersible *Nautile.* Samples were then placed in the submersible insulated basket for the trip to the surface. On board, sediments were directly inoculated in basal medium. For enrichment and isolation, the basal medium contained (per liter of distilled water) 0.3 g KH_2PO_4, 0.3 g K_2HPO_4, 1 g NH_4Cl, 23 g NaCl, 0.1 g KCl, 0.1 g $CaCl_2.2H_2O$, 0.5 g cysteine-HCl, 1 g yeast extract (Difco Laboratories), 1 ml trace mineral element solution (Widdel and Pfennig, 1981) and 1 ml of 0.1 % resazurin (Sigma). The pH was adjusted to 7.2 with a 10 M KOH solution. The medium was boiled under a stream of O_2-free N_2 gas and cooled at room temperature. Aliquots of 5 ml were dispensed into Hungate tubes, degassed under N_2-CO_2 (80/20, v/v) and subsequently sterilized by autoclaving at 120 °C for 20 min. Prior to culture inoculation, 0.1 ml of 10 % (w/v) $NaHCO_3$, 0.1 ml of 2 % (w/v) Na_2S. $9H_2O$, 0.1 ml of 150g/l $MgCl_2$. $6H_2O$ and 20 mM of glucose from sterile stock solutions were injected into the tubes. The Hungate technique (Hungate, 1969) was used throughout the study. A 0.5 aliquot of the sample was inoculated into the Hungate tubes that were subsequently incubated at 37 °C. To obtain pure cultures, the enrichment was subcultured several times under the same growth conditions prior to isolation. For isolation, the culture was serially diluted tenfold in roll tubes (Miller and Wolin, 1974) containing the same culture medium supplemented with 2 % agar (w/v). Several colonies developed after incubation at 37°C and were picked separately. Colonies were white and circular with diameters ranging from 1.0 to 2.0 mm after 3-5 days of incubation at 37 °C. The process of serial dilution was repeated several times until the isolates were deemed to be axenic. Several strains were isolated and had high similarities (> 99 %) in the 16S rRNA gene. One strain, designated Ra1766H^T, was selected for further taxonomic characterization.

Methods for purification of the DNA, PCR amplification and sequencing of the 16S rRNA gene were described previously (Ben Dhia Thabet *et al.,* 2004). The partial sequences generated were assembled using BioEdit v. 5.0.9. (Hall, 1999) and the consensus sequence of

116 1456 nucleotides was corrected manually for errors and deposited in the GenBank database
117 under accession number KC329523. The sequence was compared with available sequences in
118 GenBank, using a BLAST search (Altschul *et al*., 1990). Nucleotide ambiguities were omitted
119 and evolutionary distances were calculated using the maximum composite likelihood model
120 (Tamura et al., 2004). Phylogenetic tree topology determined using neighbor-joining (NJ)
121 method is shown in Fig. 2 (Saitou & Nei, 1987). Support for internal nodes was assessed by
122 the bootstrap analysis (1500 replicates) (Felsenstein, 1985). These analyses were conducted in
123 MEGA6 (Tamura et al., 2013). The position of strain Ra1766T related to *Geosporobacter*
124 *subterraneus* and *Thermotalea metallivorans* was also confirmed using the minimum
125 evolution method (ME) with the maximum composite likelihood model (Rzhetsky A. & Nei
126 M., 1992), and maximum likelihood method (ML) with the general time reversible model
127 (Nei M. & Kumar S., 2000). The 16S rRNA sequence analysis indicated that strain Ra1766H^T
128 was a member of the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales*, family
129 *Clostridiaceae* (Collins *et al.,* 1994). The most closely phylogenetic related species were
130 members of cluster XI of the order *Clostridiales*. They included *Geosporobacter subterraneus*
131 VNs68^T isolated from a deep geothermal aquifer (Klouche *et al.,* 2007) with 94.2 % identity,
132 *Clostridium caminithermale* DVird3^T isolated a deep-sea hydrothermal vent chimney
133 (Brisbarre *et al.,* 2003) with 93.7 % identity, and *Thermotalea metallivorans* B2-1^T isolated
134 from a borehole in the Great Artesian Aquifer (Ogg & Patel 2009) (Ogg & Patel 2009) with
135 92.4 % identity.

136 Growth experiments were performed in duplicate, using Hungate tubes containing the
137 basal medium. We also performed these experiments (growth optima determination) on the
138 two most closely-related strains in parallel, namely *Geosporobacter subterraneus* VNs68^T and
139 *Clostridium caminithermale* DVird3^T. The pH, temperature, and NaCl concentration ranges
140 for growth were determined using basal medium. Water baths were used for incubating

bacterial cultures from 20 °C to 55 °C, with increments of 5 °C. The pH of the medium was adjusted by injecting in Hungate tubes aliquots of anaerobic stock solutions of 1 M HCl (acidic pHs), 10 % NaHCO3 or 8 % Na_2CO_3 (alkaline pHs) (to test pH between 5 and 8.6) and checked after autoclaving. To study the salinity requirement, NaCl was weighed directly in the tubes (0-10 %) before the medium was dispensed. The strains Ra1766H^T, VNs68^T and DVird3^T were subcultured at least twice under the same experimental conditions before the growth rates were determined. Arabinose, cellobiose, sorbose, glucose, fructose, galactose, ribose, glycerol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, saccharose, xylose, peptone, casaminoacids, acetate, succinate, butyrate, formate, lactate and pyruvate, were tested as growth substrates during strain Ra1766H^T cultivation. Each substrate was added to the basal medium at a final concentration of 20 mM whereas H_2/CO_2 (80/20) in the presence of acetate (2 mM acetate), was tested at 2 bars. Elemental sulfur (1 % w/v), sodium sulfate (20 mM), sodium thiosulfate (20 mM), sodium sulfite (2 mM), fumarate (20 mM), sodium nitrate (10 mM), sodium nitrite (2 mM) and $Fe(OH)_3$ (13 mM) were tested as terminal electron acceptors in the presence of glucose (20 mM) and yeast extract (1 g/l). H_2S production was determined photometrically as colloidal CuS by using the method of Cord-Ruwisch (1985). Bacterial growth was monitored by measuring the increase in turbidity at 580 nm by insertion of Hungate tubes into the cuvette holder of a spectrophotometer (Cary 50, Varian). End products of metabolism were measured by high pressure liquid chromatography (HPLC) and gas chromatography of the gases released after 2 weeks of incubation at 37 °C (Fardeau *et al.,* 2000). Cellular morphology and purity of the strains were assessed under an Optiphot (Nikon) phase contrast microscope. For transmission electron microscopy studies, cells were negatively stained with sodium phosphotungstate, as previously described (Fardeau *et al.,* 1997). The presence of spores was checked by microscopic observation of cultures and pasteurization tests performed at 80, 90 and 100 °C

for 10 and 20 min. Cells of strain Ra1766H^T stained Gram-positive (Gram staining reaction). They were motile. Strain Ra1766H^T produced thin, long (0.5-1µm x 6-10µm), straight or curved rods. The presence of terminal endospores was observed only in old (more than one month incubation) cultures (Fig. 1). Ultrathin sections obtained as described by Fardeau et al. (1997) revealed a thin stratified, Gram-positive type of cell wall (Fig. 1). Phenotypic characteristics of strain Ra1766H^T are listed in Table 1 and in the genus and species descriptions as well. Strain Ra1766H^T required yeast extract for growth (1 g/l), which could be replaced by biotrypcase but not by Balch vitamins solution (Balch *et al.,* 1979). It grew at temperatures between 25 °C and 45 °C, with an optimum at 30 °C (no growth at 20 °C and 50 °C). Growth occurred at salinities between 5 g/l and 60 g/l, with an optimum at 30 g/l (no growth at 0 g/l or 65 g/l). During strain Ra1766H^T cultivation, the following carbohydrates were used as carbon and energy sources: glucose, mannose, acetate, propionate, succinate and pyruvate. Fructose, cellobiose, sorbose, ribose, xylose, methanol, formate, lactate, ethanol and H_2/CO_2 (80/20) in the presence of acetate (2 mM acetate) were not used. The main fermentation products from glucose were acetate, butyrate, ethanol, H_2 and CO_2. They were acetate, butyrate, H_2 and CO_2 from pyruvate, and acetate, butyrate, propionate, H_2 and CO_2 from succinate. Casamino acids and amino acids (glutamate, aspartate and glycine) were also fermented, whereas alanine, phenylalanine, leucine, glutamine, tyrosine, cysteine, lysine, arginine, proline, serine, isoleucine, asparagine, valine, histidine, methionine and tryptophane were not. Fatty acids were extracted using the method of Miller (1982) with the modifications of Kuykendall *et al.* (1988) and analysed by gas chromatography (model 6890N, Agilent Technologies) using the Microbial Identification System (MIDI, Sherlock Version 6.1; database, ANAER6). Analyses of the respiratory quinones and polar lipid were carried out at the Identification Service of the DSMZ (Braunschweig. Germany). The G+C content was

190 determined by using HPLC (Mesbah *et al.,* 1989) after DNA having been isolated and
191 purified by chromatography on hydroxyapatite using the procedure of Cashion *et al.* (1977).
192 The major fatty acids were $C_{14:0}$ (32.5 %), $C_{16:1}$ ω7 (21.7 %), $C_{16:1}$ ω7 DMA (10.9 %) and
193 $C_{16:0}$ (10.3 %). No quinones were detectable and the polar lipid profile of strain Ra1766H^T
194 consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine, and
195 phospholipids (supplementary Figure S1). The G+C content of the genomic DNA of strain
196 Ra1766H^T was 33.7 %.
197 Phylogenetic characteristics of strain Ra1766H^T pertaining to the cluster XI of the
198 *Clostridiales* suggests this isolate to represent a new genus within this cluster. This is strongly
199 supported by significant genetic and phenotypic traits, as detailed in Table 1. As an example,
200 strain Ra1766H^T shows values in the DNA G + C content (33.7 mol %) far from the values
201 exhibited by *Geosporobacter subterraneus* (44.2 mol %), *Thermotalea metallivorans* (48 mol
202 %) or *Caminicella sporogenes* (24.2 mol %). In contrast to *Clostridium caminithermale* which
203 exhibits a similar value in the DNA G+C content (33.1 mol %), strain Ra1766H^T used a
204 different range of substrates and differed in its metabolism by the end products of glucose
205 fermentation (Table 1). There are also some differences between all these isolates regarding
206 temperature optima for growth, strain Ra1766H^T exhibiting the lower temperature optimum
207 (Table 1). It is noteworthy that strain Ra1766H^T is slightly halophilic, thus possibly
208 confirming its marine origin. However, its ecological significance in the Guaymas Basin
209 needs further investigations, as the most closely-related strains *G. subterraneus*, *C.*
210 *caminithermale, C. sporogenes* and *T. metallivorans* share a deep origin. On the basis of the
211 overall 16S rRNA gene dissimilarity values between strain Ra1766H^T and the closest
212 described relatives as well as the phenotypic differences, we propose that strain Ra1766H^T
213 should be assigned to a new genus and a new species within cluster XI of the *Clostridium*
214 subphylum, *Crassaminicella profunda* gen. nov., sp., nov.

Description of *Crassaminicella* gen. nov.

Cras.sa.mi.ni.cel'la. L.n crassamen -inis, sediment; L. fem. n. cella, a storeroom, chamber and, in biology, a cell; N.L. fem. n. Crassaminicella, cell isolated from sediments.

Cells stain Gram-positive. They are motile, straight or curved rods occurring singly forming terminal endospores. Mesophilic, neutrophilic and slightly halophilic. Anaerobic and heterotrophic, fermenting carbohydrates and proteinaceous substrates. The end products from glucose fermentation are acetate, butyrate and ethanol with hydrogen and carbon dioxide. The genus is phylogenetically included in cluster XI of the order *Clostridiales.* The type species is *Crassaminicella profunda.*

Description of *Crassaminicella profunda* gen. nov. sp. nov.

Crassaminicella profunda. (pro.fun'da. L. fem. adj. *profunda* from the deep). Isolated from sediments of the Guaymas basin.

Description as for the species**.**

Rods are 0.5-1 μm by 6-10 μm. Growth at NaCl concentrations ranging from 0.5 to 6 %, with an optimum at 3 %. Optimal growth at pH value of 7.5 (range 6.7-8.1; no growth at pH 6.5 and 8.2). Optimal temperature for growth is 30 °C (range 25-45 °C, no growth at 20 or 50 °C).

Carbohydrates (glucose and mannose) and organic acids (pyruvate and succinate) are fermented. Casamino acids and some amino acids (glutamate, aspartate, and glycine) serve as growth substrates. The main end products of glucose catabolism are acetate, butyrate, ethanol, H_2 and CO_2. Fructose, cellobiose, sorbose, ribose, xylose, methanol, formate, lactate, fumarate, ethanol, a gas mixture of hydrogen and carbon dioxide (80:20 vol/vol) supplemented with acetate, alanine, phenylalanine, leucine, glutamine, tyrosine, cysteine, lysine, arginine, proline, isoleucine, asparagine, valine, histidine, methionine, tryptophane, threonine, and serine are not used. Sulfate, sulfite, thiosulfate and elemental sulfur are not

utilized as electron acceptors. Yeast extract is required for growth. The G+C content of genomic DNA is 33.7 mol %. The type strain is Ra1766H^T (=DSM 27501^T = JCM 19377^T), isolated from sediments of the Guaymas basin.

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References:

Alain, K., Pignet, P., Zbinden, M., Quillevere, M., Duchiron, F., Donval, J. P., Lesongeur, F., Raguenes, G., Crassous, P. & other authors (2002). *Caminicella sporogenes* gen. nov., sp. nov., a novel thermophilic spore-forming bacterium isolated from an East-Pacific Rise hydrothermal vent. *Int J Syst Evol Microbiol* **52***,* 1621–1628.

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.

BenDhia Thabet, O., Fardeau, M-L, Joulian, C., Thomas, P., Hamdi, M., Garcia, J-L. & Ollivier, B. (2004). *Clostridium tunisiense* sp. nov., a new proteolytic, sulfur-reducing bacterium isolated from an olive mill wastewater contaminated by phosphogypse. *Anaerobe* **10,** 185-190.

Brisbarre, N., Fardeau, M-L., Cueff, V., Cayol, J-L., Barbier, G., Cilia, V., Ravot, G., Thomas, P., Garcia, J-L. & Olliver, B. (2003). *Clostridium caminithermale* sp. nov.*,* a slightly halophilic and moderately thermophilic bacterium isolated from an Atlantic deep-sea hydrothermal chimney. *Int J Syst Evol Microbiol* **53***,* 1043–1049.

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 the determination of dissolved and precipitated sulfides in cultures of sulfate-reducing bacteria. *J Microbio Methods* **4,** 33-36.

Dick, G.J., Lee, Y.E. & Tebo, B.M. (2006). Manganese(II)-oxidizing Bacillus spores in Guaymas Basin hydrothermal sediments and plumes. *Appl Environ Microbiol.* **72,** 3184-3190.

Dick, G.J. & Tebo, B.M. (2010). Microbial diversity and biogeochemistry of the Guaymas Basin deep-sea hydrothermal plume. *Environ Microbiol.* **12,** 1334-1347.

Fardeau, M-L., Ollivier, B., Patel, B.K.C., Magot, M., Thomas, P., Rimbault, A., Rocchiccioli, F. & Garcia, J-L. (1997). *Thermotoga hypogea* sp. nov., a xylanolytic, thermophilic bacterium from an oil-producing well. *Int J Syst Evol Microbiol* **47,** 1013-1019.

Fardeau, M-L., Magot, M., Patel, B. K. C., 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.

Fendrich, C., Hippe, H. & Gottschalk, G. (**1990**). *Clostridium halophilium* sp. nov. and *C. litorale* sp. nov., an obligate halophilic and a marine species degrading betaine in the Stickland reaction. *Arch Microbiol* **154**, 127–132.

Gundersen, J. K., Jorgensen, B. B., Larsen, E. & Jannasch, H. W. (1992). Mats of giant sulphur bacteria on deep-sea sediments due to fluctuating hydrothermal flow *Nature* **360**, 454 – 456.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.

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

Klouche, N., Fardeau, M-L., Lascourreges, J-F., Cayol, J-L., Hacine, H., Thomas, P. & Magot, M. (**2007**). *Geosporobacter subterraneus* gen. nov., sp. nov., a spore-forming bacterium isolated from a deep subsurface aquifer. *Int J Syst Evol Microbiol* **57**, 1757–1761.

Kuykendall, L. D., Roy, M. A., O´Neil, J. J., & Devine, T. E. (1988). Fatty acids, antibiotic resistance, and desoxyribonucleic acid homology groups of *Bradyrhizobium japonicum. Int J Syst Bacteriol* **38**, 358–361.

Lakhal, R., Pradel, N., Postec, A., Hamdi, M., Ollivier, B., Godfroy, A. & Fardeau, M-L. (2013). Characterization of *Vallitalea guaymasensis* gen. nov., sp. nov., a novel marine bacterium, isolated from sediments of Guaymas basin. *Int J Syst Evol Microbiol* **63**, 3019-23.

Lupton, J. E. (1979). Helium-3 in the Guaymas Basin: Evidence for injection of mantle volatiles in the Gulf of California. *J. Geophys. Res.,* **84,** 7446-7452.

Miller, T. L., & Wolin, M.J. (1974). A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. *Appl Microbiol* **27,** 985-987.

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.

Nei, M. & Kumar, S. (2000). Molecular evolution and phylogenetics. *Oxford University Press,* New York. 333 pp.

Ogg, C. D. & Patel, B. K. C. (2009). *Thermotalea metallivorans* gen. nov., sp. nov., a thermophilic, anaerobic bacterium from the Great Artesian Basin of Australia aquifer. *Int J Syst Evol Microbiol* **59***,* 964–971.

Pearson, A., Seewald, J. S. & Eglinton, T.I. (2005). Bacterial incorporation of relict carbon in the hydrothermal environment of Guaymas Basin *Geochim. Cosmochim. Acta*, **69,** 5477-5486.

Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Mol Biol Evol* **9**, 945-967.

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

Simoneit, B. R. T. (1985). Hydrothermal petroleum-genesis, migration and deposition in Guaymas Basin, Gulf of California. *Can. J. Earth Sci.* **22,** 1919–1929.

Tamura, K., Nei, M., and Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc Natl Acad Sci (USA)* **101,** 11030-11035.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. (2013). MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol* **30**, 2725-2729.

Teske, A., Hinrichs, K. U., Edgcomb, V., de Vera Gomez, A., Kysela, D., Sylva, S. P., Sogin, M. L. & Jannasch, H. W. (2002). Microbial diversity of hydrothermal sediments in the Guaymas Basin: evidence for anaerobic methanotrophic communities. *Appl Environ Microbiol* **68**, 1994-2007.

Widdel, F. & Pfennig, N. (1981). Studies on dissimilatory sulphate reducing bacteria that decompose fatty acids. I. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments. Description of *Desulfobacter postgatei* gen. nov., sp. nov. *Arch Microbiol* **129**, 395-400.

Williams, D. L., Becker, K., Lawver, L. A., & von Herzen, R. P. (1979). Heat flow at the spreading centers of the Guaymas Basin, Gulf of California. *J. Geophys. Res.* **84**, 6757-6769.

Table 1: Differential characteristics between strain Ra1766H^T and related members of cluster XI of the order *Clostridiales* (Collins *et al.,* 1994).

Characteristics	Strain Ra1766H^T	*G. subterraneus* VNs68^T	*C. caminithermale* DVird3^T	*C. sporogenes* AM1114^T	*T. metallivorans* B2-1^T
Presence of spores	+	+	+	+	-
Gram type	+	+	+	-	-
Temperature growth range (optimum) (°C)	25-45 (30)	25-55 (45)*	20-60 (45)*	45-65 (60)	30-55 (50)
pH growth range	6.7-8.1 (7.5)	5.6-8.5 (7.3)*	5.8–8.6 (6.6)*	4.5-8 (7.5)	6.5-9 (8)
NaCl growth range (g.L^{-1})	5-60 (30)	0-45 (20)*	10-45 (35)*	15-46 (19-23)	0-40 (0-10)
Motility	+	-	+	+	+
Substrates used					
Cellobiose	-	+	-	ND	+
fructose	-	+	+	+	+
succinate	+	-	+	+	-
propionate	-	-	-	ND	-
pyruvate	+	-	+	-	-
ribose	-	+	+	ND	-
mannose	+	-	+	ND	+
Reduction of Fe(III)	-	+*	-*	-	ND
End products of glucose fermentation**	Acetate, butyrate, ethanol, H_2, CO_2	Acetate, formate, H_2, CO_2, (ethanol)	Acetate, butyrate, propionate, H_2, CO_2	Butyrate, acetate, ethanol, H_2, CO_2	Ethanol, acetate
DNA G + C content (mol %)	33.7	42.2	33.1	24.2	48

* this study

** trace products in parentheses

Figure legends:

Figure 1. Thin-section electron microphotograph showing the cell morphology of strain Ra1766H^T and Gram-positive type of cell wall A, bar: 0.2 µm. Insert : optical microphotograph showing the morphology and the presence of terminal endospore.

Figure 2. Neighbour-joining phylogenetic tree of the 16S rRNA gene sequence showing the relationships between strain Ra1766H^T and related type strains among cluster XI of the order *Clostridiales*. *Caloranaerobacter azorensis* MV1087^T belonging to the cluster XII was used as outgroup. Bootstrap values >70% based on 1500 replicates are shown. Bar, 1 change per 100 nucleotide positions.

Figure
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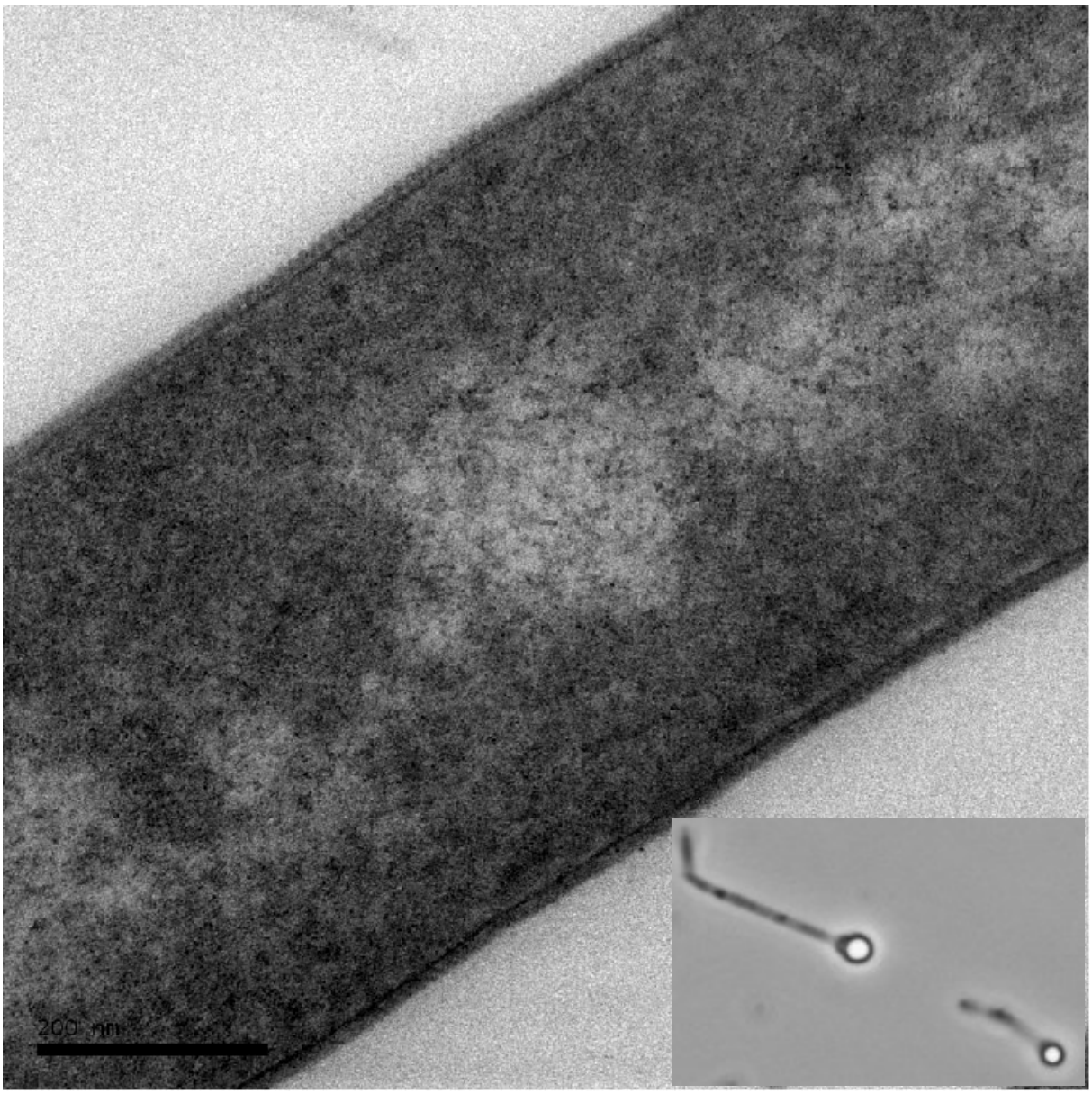

Figure 1.

Figure
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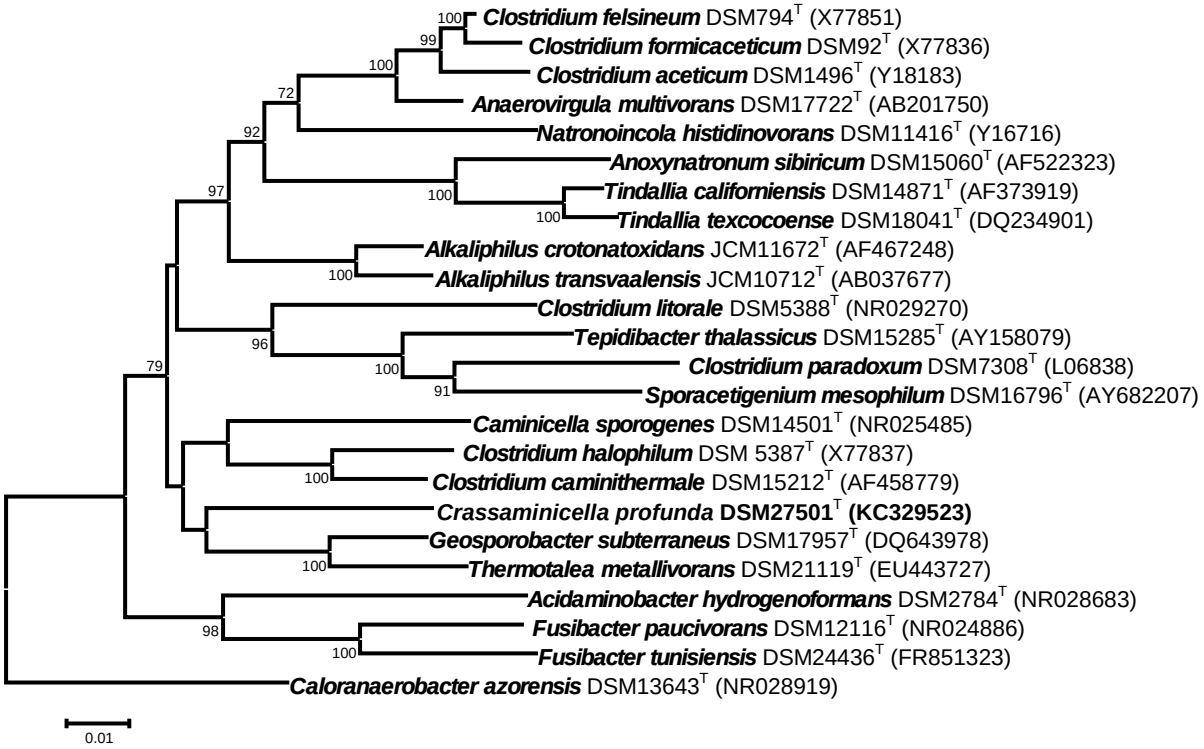

Figure 2.

Supplementary Material Files

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