International Journal of Systematic and Evolutionary Microbiology (2003), **53**, 1043–1049 **DOI** 10.1099/ijs.0.02471-0

Clostridium caminithermale sp. nov., a slightly halophilic and moderately thermophilic bacterium isolated from an Atlantic deep-sea hydrothermal chimney

Nadège Brisbarre,[1] Marie-Laure Fardeau,[1] Valérie Cueff,[2] Jean-Luc Cayol,[1] Georges Barbier,[2] Valérie Cilia,[3] Gilles Ravot,[3] Pierre Thomas,[1] Jean-Louis Garcia[1] and Bernard Ollivier[1]

Correspondence
Bernard Ollivier
ollivier@esil.univ-mrs.fr

[1]IRD, UR 101 Extrêmophiles, IFR-BAIM, Universités de Provence et de la Méditerranée, ESIL, Marseille, France

[2]IFREMER, Laboratoire de Microbiologie et de Biotechnologie des Extrêmophiles, Plouzané, France

[3]Protéus S.A., Nîmes, France

A strictly anaerobic, slightly halophilic and moderately thermophilic, sporulating rod designated strain DVird3^T was isolated from deep-sea hydrothermal vent samples collected at a depth of approximately 800 m on the Atlantic Ocean Ridge. Strain DVird3^T possessed a few laterally inserted flagella, had a DNA G+C content of 33·1 mol% and grew optimally at pH 6·6 and at 45 °C. Growth was observed at temperatures between 20 and 58 °C and at pH values between 5·8 and 8·2. The optimum NaCl concentration for growth was 3 % sea salt (30 g l^{-1}); no growth was observed in the presence of 15 or 60 g sea salt l^{-1}. Strain DVird3^T is heterotrophic and utilizes some sugars and various single amino acids. Acetate was the main fatty acid detected from carbohydrate fermentation, together with H_2 and CO_2. Gelatin was used as an energy source. It performed the Stickland reaction. Phylogenetically, strain DVird3^T branched with members of cluster XI of the order *Clostridiales*, with *Clostridium halophilum* as its closest relative (similarity of 94·6 %). On the basis of its phenotypic, genotypic and phylogenetic characteristics, strain DVird3^T (=DSM 15212^T=CIP 107654^T) is proposed as the type strain of a novel species of the genus *Clostridium*, *Clostridium caminithermale* sp. nov.

Deep-sea hydrothermal vents are inhabited by a wide range of microbial communities that comprise free-living micro-organisms as well as micro-organisms living in association with invertebrates (Jeanthon, 2000). Among these free-living micro-organisms are bacteria belonging to the ε-subclass of the *Proteobacteria*, and the significance of their ecological role has been identified, mainly in molecular surveys (Haddad *et al.*, 1995; Jeanthon, 2000; Reysenbach *et al.*, 2000b; Corre *et al.*, 2001). The recent isolation, from deep-sea hydrothermal vents, of moderately thermophilic and thermophilic members of the ε-subclass of the *Proteobacteria* has demonstrated the ability of these micro-organisms to grow autotrophically using elemental sulfur as a terminal electron acceptor (Campbell *et al.*, 2001; Alain *et al.*, 2002a). This trait of sulfur reduction has also been found to be a common physiological feature of the well-studied, anaerobic, chemoautotrophic, thermophilic and hyperthermophilic micro-organisms (domain *Archaea*) isolated from these peculiar deep marine environments (Jeanthon, 2000). Besides chemoautotrophy and mixotrophy, chemoheterotrophy has also been recognized as a significant type of metabolism within the deep-sea hydrothermal vent anaerobic microbial community (Jeanthon, 2000). Among such trophic microbial groups inhabiting deep environments, little attention has been paid to anaerobic mesophiles and moderate thermophiles in comparison with thermophilic and hyperthermophilic micro-organisms from both domains (*Bacteria* and *Archaea*). Therefore, most microbiological studies performed so far have underlined the fact that thermophilic and hyperthermophilic archaea thrive in the hottest part of the ecosystem, with only a few thermophilic anaerobic members of the domain *Bacteria* being isolated (Jeanthon, 2000). The latter include members of the orders *Thermotogales* and *Clostridiales*. The order *Clostridiales* includes two thermophilic isolates: one, *Caloranaerobacter azorensis* (Wery *et al.*, 2001), is from the Atlantic rise hydrothermal vent; the other, *Caminicella*

The GenBank accession number for the 16S rRNA gene sequence of strain DVird3^T is AF458779.

sporogenes (Alain *et al.*, 2002b), is from the East-Pacific Rise hydrothermal vent. They respectively belong to clusters XII and XI of the *Clostridium* subphylum. Here, we report the isolation and characterization of a novel moderately thermophilic member of cluster XI of the *Clostridium* subphylum, originating from a deep-sea hydrothermal vent. We propose to assign this novel bacterium to a novel species of the genus *Clostridium*, *Clostridium caminithermale* sp. nov.

Samples of an active chimney from a North Atlantic Ocean deep-sea hydrothermal vent (Diva2 cruise, Menez Gwen site, 37°50′N, 31°31′W) were collected on 20 June 1994 at a depth of approximately 800 m, using the submarine *Nautile*. The samples were crushed anaerobically on board in sterile sea water in an anaerobic chamber. The resulting mud was transferred (i) into 50 ml serum vials closed with butyl rubber stoppers for storage at 4 °C, and (ii) into 1·8 ml cryotubes containing 90 µl DMSO as cryoprotectant for storage at −20 °C until processing. The Hungate technique (Hungate, 1969) was used throughout this study. The basal medium (BM) contained the following (l^{-1} distilled water): 1 g NH_4Cl, 0·3 g K_2HPO_4, 0·3 g KH_2PO_4, 30 g sea salt (Sigma), 0·5 g cysteine hydrochloride, 1 mg resazurin (Sigma), 0·5 g peptone, 0·5 g yeast extract (Fisher Scientific) and 10 ml trace mineral element solution (Balch *et al.*, 1979). The pH was adjusted to 7 with 10 M KOH. The medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature. Five-millilitre aliquots were dispensed into Hungate tubes and 20 ml aliquots were dispensed into serum bottles under a stream of N_2/CO_2 (80 : 20, v/v), and the sealed vessels were then autoclaved for 45 min at 110 °C. Prior to inoculation, $Na_2S.9H_2O$ and $NaHCO_3$ were injected from sterile stock solutions to respective final concentrations of 0·04 and 0·2 % (w/v). Enrichment was performed in 120 ml serum bottles. Xylan 'oat spelt' (2·5 g l^{-1}) and xylan 'birchwood' (2·5 g l^{-1}) were added to the BM as an electron donor, with thiosulfate (20 mM) as an electron acceptor. The serum bottles were each inoculated with 6 ml sample, corresponding to approximately 10 % (v/v) of the final liquid volume. The bottles were incubated at 50 °C in a controlled-temperature oven for 2–3 days. Isolation was performed in the same medium. Three enrichment series were performed before isolation.

Growth experiments were performed in duplicate, using Hungate tubes containing BM. For pH growth experiments, BM containing glucose (20 mM) was adjusted to different pH values by injecting $NaHCO_3$ or Na_2CO_3 from 10 % (w/v) sterile anaerobic stock solutions and then incubated at 45 °C. The temperature range for growth was determined using the same medium and adjusted to the optimum pH for growth. For studies on sea-salt requirements, sea salt was weighed directly into Hungate tubes and BM without sea salt was adjusted at the optimum pH for growth and dispensed into tubes as described above. Tubes were incubated at 45 °C. The strain was subcultured at least once under the same experimental conditions prior to inoculation for growth experiments. The presence of spores was determined by microscopic examination of the culture at different phases of growth. For substrate-utilization tests, BM was used. The substrates were injected from stock solutions into Hungate tubes to a final concentration of 10 mM for amino acids (L-leucine was the only amino acid weighed directly into the Hungate tubes), 5 g l^{-1} for peptides and proteins and 20 mM for sugars, fatty acids and alcohols. To test for sulfur-containing electron acceptors, thiosulfate (20 mM), sulfate (20 mM), elemental sulfur (2 %) and sulfite (2 mM) were added to the growth medium. Nitrate (10 mM) and nitrite (10 mM) were also tested as potential electron acceptors. Light and electron microscopy were performed as described previously (Cayol *et al.*, 1994). Growth was measured by inserting duplicate Hungate tubes directly into a model UV-160A spectrophotometer (Shimazu) and measuring the OD_{580}. Hydrogen and fermentation products (alcohols, volatile and non-volatile fatty acids) were quantified as described previously (Fardeau *et al.*, 1993). Amino acid concentrations were determined by HPLC (Moore *et al.*, 1958). The G+C content of the DNA and DNA–DNA hybridization were determined at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, as described by Hernández-Eugenio *et al.* (2002).

The methods for the purification and extraction of DNA and the amplification and sequencing of the 16S rRNA gene have been described previously (Andrews & Patel, 1996; Love *et al.*, 1993; Redburn & Patel, 1993). Samples were loaded onto an Applied Biosystems 373XL sequencer and run for 12 h on a 4·5 % denaturing acrylamide gel. Sequence data were imported into the sequence editor BioEdit version 5.0.9 (Hall, 1999); the base calling was examined and a contiguous consensus sequence was obtained for each isolate. The full sequence was aligned using the SEQUENCE ALIGNER program of the Ribosomal Database Project (RDP; Maidak *et al.*, 2001). The consensus sequence was then adjusted manually to conform to the 16S rRNA secondary-structure model (Winker & Woese, 1991). A non-redundant BLAST search (Altschul *et al.*, 1997) of the full sequence through GenBank identified its closest relative. Sequences used in the phylogenetic analysis were obtained from the RDP (Maidak *et al.*, 2001) and GenBank (Benson *et al.*, 1999). Positions of sequence and alignment ambiguity were omitted and the pairwise evolutionary distances based on 1263 unambiguous nucleotides were calculated using the method of Jukes & Cantor (1969). Dendrograms were constructed using the neighbour-joining method (Saitou & Nei, 1987). Confidence in the tree topology was determined by using 100 bootstrapped trees (Felsenstein, 1985).

Enrichment cultures were incubated at 50 °C for 1 week. Growth was regarded as positive on the basis of optical density. Microscopic examination of the enrichment culture revealed the presence of large rods. Brown, circular colonies, 1–2 mm in diameter, appeared after 3 days incubation at 50 °C in roll tubes containing basal agar medium. Single colonies were picked using the techniques developed by

Hungate (1969), and the process of serial dilution in roll tubes was repeated at least twice to purify the cultures. Six rod-shaped bacteria were isolated, designated strains DVird1–DVird6. Analysis of partial 16S rRNA gene sequences (200 bp) of the six strains showed that they were closely related phylogenetically, with a degree of similarity close to 100 % (data not shown). Strain DVird3^T was further characterized.

Strain DVird3^T was a strictly anaerobic rod. The cells were 0·4–0·5 μm in width and 5–9 μm in length and occurred singly or in pairs. Cells were motile by means of a few laterally inserted flagella. Spore formation was induced in the presence of oxygen and vitamins. Spores were oval and subterminal (Fig. 1a). Electron microscopy of sections of strain DVird3^T exhibited a thick, stratified, Gram-positive-type cell wall composed of three dense layers (two thick layers and a thinner middle layer) separated by two less dense spaces (Fig. 1b). Strain DVird3^T was moderately thermophilic and grew at temperatures ranging from 20 to 58 °C, with an optimum at 45 °C. The isolate was slightly halophilic and grew in the presence of sea-salt concentrations ranging from 12 to 55 g l^{-1}, with an optimum at 30 g l^{-1}. Strain DVird3^T was neutrophilic; the optimum pH for growth was 6·6 and growth occurred between pH 5·8 and 8·2. In optimal conditions (batch conditions), the OD_{580} can reach 1·0.

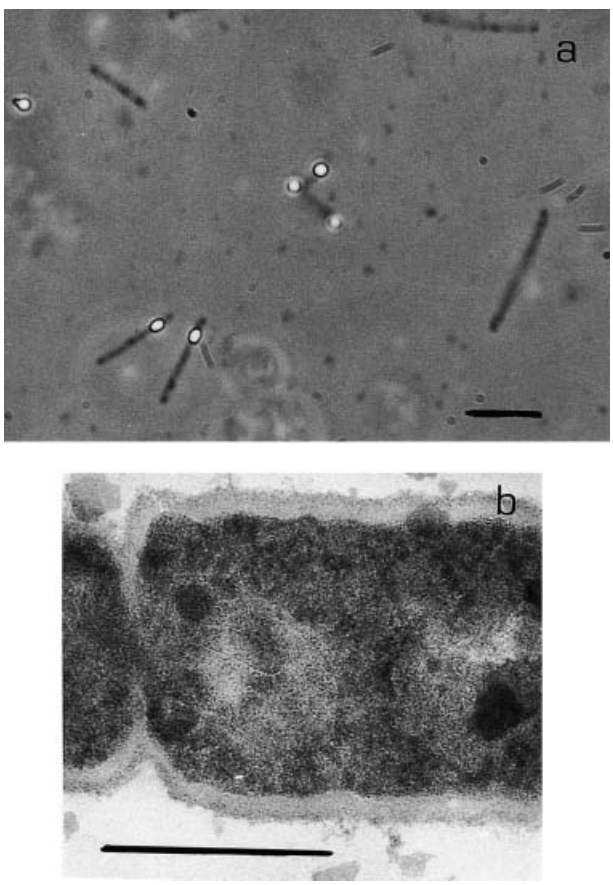

Fig. 1. (a) Phase-contrast micrograph of cells of strain DVird3^T, showing subterminal oval spores swelling the sporangia. Bar, 5 μm. (b) Electron micrograph of an ultrathin section of strain DVird3^T, showing the Gram-positive cell-wall structure composed of three dense layers (two thick layers and a thinner one in the middle), separated by two less dense spaces. Bar, 0·25 μm.

Yeast extract was required for growth on sugars. Strain DVird3^T fermented yeast extract, peptone, Bio-trypticase and Casamino acids into a mixture of volatile fatty acids (acetate, propionate, butyrate, isobutyrate, isovalerate). Fructose, galactose, glucose, glycerol, maltose, mannose and ribose were used as energy sources. Glucose was converted into acetate [1·2 mol (mol glucose consumed)$^{-1}$], butyrate [0·2 mol (mol glucose consumed)$^{-1}$], propionate [0·2 mol (mol glucose consumed)$^{-1}$] and H_2+CO_2. Propionic acid was the only fatty acid detected from succinate fermentation, whereas acetate together with butyrate and propionate were end-products of pyruvate metabolism. The following substrates were not used: arabinose, cellobiose, lactose, mannitol, melibiose, raffinose, rhamnose, starch, sucrose, xylose, dulcitol, sorbitol, lactate, formate, acetate, propionate and H_2+CO_2. Use of amino acids as energy sources is detailed in the species description below. Strain DVird3^T performed the Stickland reaction, using isoleucine as electron donor and methionine or betaine as electron acceptors. Other properties are detailed in the species description. The G+C content of strain DVird3^T was 33·1 mol% (HPLC).

16S rRNA sequence analysis revealed that strain DVird3^T was a member of cluster XI of the *Clostridium* subphylum, *Clostridium halophilum* being its closest phylogenetic relative (94·6 % similarity). Fig. 2 presents a dendrogram generated by the neighbour-joining method (Felsenstein,

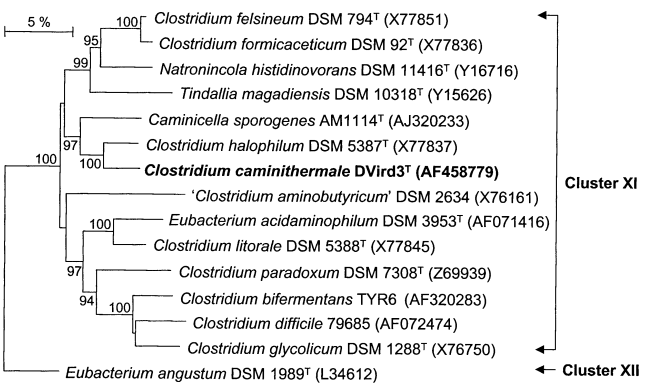

Fig. 2. Phylogenetic dendrogram, based on 16S rDNA sequence data, indicating the position of strain DVird3^T amongst cluster XI of the order *Clostridiales* and related bacteria. The clusters are defined on the basis of the guidelines described by Collins *et al.* (1994). Accession numbers are shown in parentheses. Bar, 5 substitutions per 100 nucleotides.

1993) from the Jukes–Cantor evolutionary similarity matrix (Jukes & Cantor, 1969). DNA–DNA hybridization between strain DVird3^T and *Clostridium halophilum* showed 50·9 % relatedness.

Both molecular and microbiological studies within deep-sea hydrothermal vent ecosystems have demonstrated the ecological significance of anaerobic and microaerophilic thermophilic to hyperthermophilic micro-organisms (domains *Bacteria* and *Archaea*), most of which are involved, in particular, in the reduction (anaerobes) or oxidation (microaerophiles) of elemental sulfur (Jeanthon, 2000; Alain *et al.*, 2002a; Götz *et al.*, 2002). Thermophilic microaerophiles are recognized as hydrogenotrophic bacteria belonging to the order *Aquificales* (Reysenbach *et al.*, 2000a; Götz *et al.*, 2002), but hydrogenotrophic anaerobic thermophiles have also been recovered from deep-sea hydrothermal environments (Jeanthon, 2000; Jeanthon *et al.*, 1998; L'Haridon *et al.*, 1998; Alain *et al.*, 2002a). The latter include methanogens, sulfate-reducing bacteria, members of the ε-subclass of the *Proteobacteria*, and heterotrophic sulfur-reducers belonging to the domain *Archaea*. It is only recently that two heterotrophic thermophilic anaerobes belonging to the order *Clostridiales* have been isolated and characterized. *Caloranaerobacter azorensis* (Wery *et al.*, 2001), isolated from a chimney sample, was ascribed as a member of cluster XII of the order *Clostridiales*, while *Caminicella sporogenes* (Alain *et al.*, 2002b), isolated from entire tube samples of *Alvinella pompejana* attached to small fragments of chimney rocks, was ascribed as a member of cluster XI of the same order as defined by Collins *et al.* (1994). Both micro-organisms are considered as thermophiles, growing at temperatures above 60 °C.

The genus *Clostridium*, order *Clostridiales*, was first defined as containing Gram-positive, anaerobic, rod-shaped and spore-forming bacteria unable to carry out the dissimilatory sulfate reduction (Cato *et al.*, 1986; Hippe *et al.*, 1992), but the genus has been reassessed taxonomically on the basis of phylogenetic considerations (Cato & Stackebrandt, 1989; Collins *et al.*, 1994; Lawson *et al.*, 1993; Rainey & Stackebrandt, 1993; Rainey *et al.*, 1993; Stackebrandt & Rainey, 1997). Most members of this genus are ubiquitous chemo-organotrophic micro-organisms that may use carbohydrates and/or proteinaceous compounds as energy sources (Cato *et al.*, 1986; Hippe *et al.*, 1992). Here, we report on the isolation and characterization of a novel member of cluster XI, order *Clostridiales*, unable to grow at 60 °C and thus considered as a moderate thermophile. Similarly to most members of this cluster, strain DVird3^T is an anaerobic, spore-forming micro-organism that grows heterotrophically on carbohydrates, peptones and amino acids. A mixture of volatile fatty acids, including formate, acetate, propionate, butyrate, isobutyrate and isovalerate, together with H_2 and CO_2, was produced from peptone fermentation by this strain. Acetate was found to be the primary fatty acid produced from glucose metabolism, with butyrate and propionate being produced in minor amounts. The 16S rRNA gene sequence analysis of this isolate indicated that it has two halophiles, *Clostridium halophilum* (similarity of 95·1 %) and *Caminicella sporogenes* (similarity of 91·8 %), as its closest phylogenetic relatives, indicating that the halophilic character of these three bacteria is an important phenotype in the assembly of these bacteria in the same phylogenetic lineage within cluster XI of the order *Clostridiales*. Besides halophiles, this cluster also contains a few other extremophilic micro-organisms including moderate thermophiles and alkaliphiles. Strain DVird3^T differs from *Clostridium halophilum* (Fendrich *et al.*, 1990) in terms of the range of sugars and amino acids used, and also in the ranges of temperature and NaCl concentration for growth (Table 1). In view of these phenotypic characteristics, *Clostridium halophilum* is a thermotolerant, moderately halophilic micro-organism, while strain DVird3^T is a moderately thermophilic, slightly halophilic micro-organism. Strain DVird3^T also differs from *Clostridium halophilum* in that its DNA has a higher G + C content (33·1 versus 26·9 mol%). In contrast to *Caminicella sporogenes*, strain DVird3^T did not grow at temperatures above 60 °C, used a wider range of sugars, did not reduce elemental sulfur or thiosulfate, performed the Stickland reaction and also had DNA with a higher G + C content (33·1 versus 24·2 mol%) (Table 1). Strain DVird3^T therefore represents a distinct phenotypic and phylogenetic lineage within cluster XI. Taking its phenotypic and phylogenetic characteristics into account, we propose to assign strain DVird3^T as the type strain of a novel species of the genus *Clostridium* (family *Clostridiaceae*, order *Clostridiales*, cluster XI), *Clostridium caminithermale* sp. nov. Strain DVird3^T is the first representative of the genus *Clostridium* that originates from deep-sea hydrothermal vents, confirming the ubiquity of members of this genus across the planet.

The isolation of *Clostridium caminithermale* extends our knowledge of the bacterial diversity inhabiting deep-sea hydrothermal environments and suggests that, besides thermophilic and hyperthermophilic anaerobic heterotrophic communities, moderately thermophilic communities must also be taken into consideration regarding the overall carbon cycle recovery in these deep ecosystems. This is also true for the moderately thermophilic hydrogenotrophic anaerobic bacteria that have recently been isolated from hydrothermal vents along the East-Pacific Rise and at the Guaymas basin (Campbell *et al.*, 2001). Interestingly, in contrast to the two other isolates from deep-sea hydrothermal vents belonging to the order *Clostridiales*, *Clostridium caminithermale* did not reduce any sulfur-containing compounds, including thiosulfate and elemental sulfur (see below), indicating that sulfur reduction might not be a common physiological trait amongst the anaerobic thermophiles that inhabit this deep ecosystem.

Description of *Clostridium caminithermale* sp. nov.

Clostridium caminithermale (ca.mi.ni.ther.ma′le. L. n. *caminus* chimney; L. pl. n. *thermae* hot springs; N.L. neut.

Table 1. Characteristics that differentiate strain DVird3^T from its closest relatives

Data were taken from this study (strain DVird3^T), Fendrich *et al.* (1990) (*Clostridium halophilum*) and Alain *et al.* (2002b) (*Caminicella sporogenes*). −, Negative; +, positive; ND, not determined; w; weak growth.

Characteristic	Strain DVird3^T	*Clostridium halophilum*	*Caminicella sporogenes*
Gram type	Positive	Positive	Negative
Temperature for growth (°C):			
Range	20–58	18–49	45–65
Optimum	45	41	55–60
pH for growth:			
Range	5·8–8·2	6·0–8·0	4·5–8·0
Optimum	6·6	7·4	7·5–8·0
Sea-salt concentration for growth (g l^{-1}):			
Range	>15–<60	20–130	20–60
Optimum	30	80	25–30
G+C content of DNA (mol%)	33·1	26·9	23·2–25·2
Stickland reaction	Positive	Positive	Negative
Reduction of S^0	−	ND	+
Reduction of thiosulfate	−	−	+
Substrates used:			
Cellobiose	−	+	−
Fructose	+	+	−
Galactose	+	ND	W
Glycerol	+	−	ND
Mannitol	−	+	ND
Dulcitol	−	+	ND
Sorbitol	−	+	−
Products of glucose fermentation:			
Ethanol	−	+	+
Propionate	+	−	−
Lactate	−	+	−

adj. *caminithermale* of a thermal chimney, describing the site of sampling).

Cells are strictly anaerobic rods, 0·4–0·5 × 5–9 μm, occurring singly or in pairs. Motile by a few laterally inserted flagella. Electron microscopy of sections of cells exhibits a thick, layered, Gram-positive-type cell wall composed of three dense layers (two thick layers and a middle thinner layer) separated by two less dense spaces. Grows at 20–58 °C (optimum 45 °C). Grows in the presence of sea salt at 12–55 g l^{-1} (optimum 30 g l^{-1}). The optimum pH for growth is 6·6, but growth occurs between pH 5·8 and 8·2. Heterotrophic. Yeast extract is required for growth on sugars. Ferments yeast extract, peptone, Bio-trypticase and Casamino acids into a mixture of volatile fatty acids. Fructose, galactose, glucose, glycerol, maltose, mannose and ribose are fermented primarily into acetate, butyrate, propionate and $H_2 + CO_2$. Succinate, fumarate and pyruvate are also fermented. The following substrates are not used: arabinose, cellobiose, lactose, mannitol, melibiose, raffinose, rhamnose, starch, sucrose, xylose, dulcitol, sorbitol, lactate, formate, acetate, propionate and $H_2 + CO_2$. The following amino acids are used as energy sources in the presence of yeast extract and peptone (0·5 g l^{-1}): arginine, cysteine, glycine, proline and tyrosine (oxidized to acetate); glutamic acid (to propionate); histidine (to propionate and formate); isoleucine (to methyl 2-butyrate); leucine (to isovalerate); lysine (to acetate and butyrate); and methionine (to propionate and acetate). The following amino acids are not used: alanine, asparagine, aspartic acid, glutamine, phenylalanine, serine, threonine, tryptophan and valine. Performs the Stickland reaction, using isoleucine as electron donor and methionine or betaine as electron acceptors. Does not use elemental sulfur, sulfate, thiosulfate, sulfite, nitrate or nitrite as an electron acceptors. The following tests were negative: β-galactosidase, arginine dihydrolase, lysine and ornithine decarboxylases, Simmons' citrate, H_2S production, urease, tryptophan deaminase, indole and acetoin production (Voges–Proskauer reaction). Adverse effects on animals and humans are not known. Because of the ability to degrade amino acids and peptides, the possibility of harmful effects cannot be excluded. Cautious handling and autoclaving of cultures before disposal is recommended. The G+C content of the type strain is 33·1 mol% (HPLC).

The type strain, strain DVird3^T (=DSM 15212^T=CIP 107654^T), was isolated from an Atlantic Ocean hydrothermal chimney.

Acknowledgements

We thank the chief scientists of the French oceanographic cruise DIVA2, Daniel Desbruyères and Anne-Marie Alayse (IFREMER, Brest, France). We thank the Captain and the crew of the N.O. *Nadir*, and the D.S.V. *Nautile* pilots and support crew. Many thanks are due to M. Giraudon-Paoli for technical assistance and C. Le Saulnier for improving the manuscript. This work was supported by the French Research Ministry (decision d'aide 00G0178).

References

Alain, K., Querellou, J., Lesongeur, F., Pignet, P., Crassous, P., Raguénès, G., Cueff, V. & Cambon-Bonavita, M.-A. (2002a). *Caminibacter hydrogeniphilus* gen. nov., sp. nov., a novel thermophilic hydrogen-oxidizing bacterium isolated from an East Pacific Rise hydrothermal vent. *Int J Syst Evol Microbiol* **52**, 1317–1323.

Alain, K., Pignet, P. Zbinden, M. & 8 other authors (2002b). *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., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Andrews, K. T. & Patel, B. K. C. (1996). *Fervidobacterium gondwanense* sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. *Int J Syst Bacteriol* **46**, 265–269.

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

Benson, D. A., Boguski, M. S., Lipman, D. J., Ostell, J., Ouellette, B. F. F., Rapp, B. A. & Wheeler, D. L. (1999). GenBank. *Nucleic Acids Res* **27**, 12–17.

Campbell, B. J., Jeanthon, C., Kotska, J. E., Luther, G. W., III & Cary, S. C. (2001). Growth and phylogenetic properties of novel bacteria belonging to the epsilon subdivision of the *Proteobacteria* enriched from *Alvinella pompejana* and deep-sea hydrothermal vents. *Appl Environ Microbiol* **67**, 4566–4572.

Cato, E. & Stackebrandt, E. (1989). Taxonomy and phylogeny. In *Clostridia*, pp. 1–26. Edited by N. P. Minton & D. J. Clarke. New York: Plenum.

Cato, E. P., George, W. L. & Finegold, S. M. (1986). Genus *Clostridium*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1141–1200. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Cayol, J.-L., Ollivier, B. Lawson, A. S. A. & 7 other authors (1994). *Haloincola saccharolytica* subsp. *senegalensis* subsp. nov., isolated from the sediments of a hypersaline lake, and emended description of *Haloincola saccharolytica*. *Int J Syst Bacteriol* **44**, 805–811.

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.

Corre, E., Reysenbach, A. L. & Prieur, D. (2001). ε-Proteobacterial diversity from a deep-sea hydrothermal vent on the Mid-Atlantic Ridge. *FEMS Microbiol Lett* **205**, 329–335.

Fardeau, M.-L., Cayol, J.-L., Magot, M. & Ollivier, B. (1993). H_2 oxidation in the presence of thiosulfate, by a *Thermoanaerobacter* strain isolated from an oil-producing well. *FEMS Microbiol Lett* **113**, 327–332.

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

Felsenstein, J. (1993). PHYLIP: phylogenetic inference package, version 3.5.1. Department of Genetics, University of Washington, Seattle, USA.

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.

Götz, D., Banta, A., Beveridge, T. J., Rushdi, A. I., Simoneit, B. R. T. & Reysenbach, A.-L. (2002). *Persephonella marina* gen. nov., sp. nov. and *Persephonella guaymasensis* sp. nov., two novel, thermophilic, hydrogen-oxidizing microaerophiles from deep-sea hydrothermal vents. *Int J Syst Evol Microbiol* **52**, 1349–1359.

Haddad, A., Camacho, F., Durand, P. & Cary, S. C. (1995). Phylogenetic characterization of the epibiotic bacteria associated with the hydrothermal vent polychaete *Alvinella pompejana*. *Appl Environ Microbiol* **61**, 1679–1687.

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.

Hernández-Eugenio, G., Fardeau, M.-L., Cayol, J.-L., Patel, B. K. C., Thomas, P., Macarie, H., Garcia, J.-L. & Ollivier, B. (2002). *Clostridium thiosulfatireducens* sp. nov., a proteolytic, thiosulfate- and sulfur-reducing bacterium isolated from an upflow anaerobic sludge blanket (UASB) reactor. *Int J Syst Evol Microbiol* **52**, 1461–1468.

Hippe, H., Andreesen, J. R. & Gottschalk, G. (1992). The genus *Clostridium* – nonmedical. In *The Prokaryotes*, vol. II, pp. 1800–1866. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K. H. Schleifer. New York: Springer.

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

Jeanthon, C. (2000). Molecular ecology of hydrothermal vent microbial communities. *Antonie van Leeuwenhoek* **77**, 117–133.

Jeanthon, C., L'Haridon, S., Reysenbach, A. L., Vernet, M., Messner, P., Sleytr, U. W. & Prieur, D. (1998). *Methanococcus infernus* sp. nov., a novel hyperthermophilic lithotrophic methanogen isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **48**, 913–919.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 211–232. Edited by H. N. Munro. New York: Academic Press.

Lawson, P. A., Perez, P. L., Hutson, R. A., Hippe, H. & Collins, M. D. (1993). Towards a phylogeny of the clostridia based on 16S rRNA sequences. *FEMS Microbiol Lett* **113**, 87–92.

L'Haridon, S., Cilia, V., Messner, P., Raguénès, G., Gambacorta, A., Sleytr, U. W., Prieur, D. & Jeanthon, C. (1998). *Desulfurobacterium thermolithotrophicum* gen. nov., sp. nov., a novel autotrophic, sulfur-reducing bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Bacteriol* **48**, 701–711.

Love, C. A., Patel, B. K. C., Nichols, P. D. & Stackebrandt, E. (1993). *Desulfotomaculum australicum* sp. nov., a thermophilic sulfate-reducing bacterium isolated from the Great Artesian Basin of Australia. *Syst Appl Microbiol* **16**, 244–251.

Maidak, B. L., Cole, J. R. Lilburn, T. G. & 7 other authors (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173–174.

Moore, S., Spackman, D. H. & Stein, W. H. (1958). Chromatography of amino acids on sulfonated polystyrene resins: an improved system. *Anal Chem* **30**, 1185–1190.

Rainey, F. A. & Stackebrandt, E. (1993). 16S rRNA analysis reveals phylogenetic diversity among the polysaccharolytic clostridia. *FEMS Microbiol Lett* **113**, 125–128.

Rainey, F. A., Ward, N. L., Morgan, H. W., Toalster, R. & Stackebrandt, E. (1993). Phylogenetic analysis of anaerobic thermophilic bacteria: aid for their reclassification. *J Bacteriol* **175**, 4772–4779.

Redburn, A. C. & Patel, B. K. C. (1993). Phylogenetic analysis of *Desulfotomaculum thermobenzoicum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *FEMS Microbiol Lett* **113**, 81–86.

Reysenbach, A. L., Banta, A., Boone, D. R., Cary, S. C. & Luther, G. W. (2000a). Microbial essentials at hydrothermal vents. *Nature* **404**, 835–836.

Reysenbach, A. L., Longnecker, K. & Kirshtein, J. (2000b). Novel bacterial and archaeal lineages from an in situ growth chamber deployed at the Mid-Atlantic Ridge hydrothermal vent. *Appl Environ Microbiol* **66**, 3798–3806.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 405–425.

Stackebrandt, E. & Rainey, F. A. (1997). Phylogenetic relationships. In *The Clostridia: Molecular Biology and Pathogenesis*, pp. 3–19. Edited by J. I. Rood, B. A. McClane, J. G. Songer & R. W. Titball. New York: Academic Press.

Wery, N., Moricet, J.-M., Cueff, V., Jean, J., Pignet, P., Lesongeur, F., Cambon-Bonavita, M.-A. & Barbier, G. (2001). *Caloranaerobacter azorensis* gen. nov., sp. nov., an anaerobic thermophilic bacterium isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol* **51**, 1789–1796.

Winker, S. & Woese, C. R. (1991). A definition of the domain *Archaea*, *Bacteria* and *Eucarya* in terms of small subunit ribosomal RNA characteristics. *Syst Appl Microbiol* **13**, 161–165.