Caldilinea tarbellica sp. nov., a filamentous, thermophilic, anaerobic bacterium isolated from a deep hot aquifer in the Aquitaine Basin

Patrick Grégoire,[1,2] Malek Bohli,[1] Jean-Luc Cayol,[1] Manon Joseph,[1] Sophie Guasco,[3] Karine Dubourg,[2] Jean Cambar,[2] Valérie Michotey,[3] Patricia Bonin,[3] Marie-Laure Fardeau[1] and Bernard Ollivier[1]

Correspondence
Bernard Ollivier
bernard.ollivier@univmed.fr

[1]Laboratoire de Microbiologie et de Biotechnologie des Environnements Chauds UMR 180, IRD, Universités de Provence et de la Méditerranée, ESIL case 925, 163 avenue de Luminy, 13288 Marseille, France

[2]Institut du Thermalisme, Université Victor Segalen Bordeaux 2, 8 rue Sainte Ursule, 40100 Dax, France

[3]LMGEM, UMR 6117, Centre d'Océanologie de Marseille, Université Aix Marseille 2 Campus de Luminy - Case 901, 163 Avenue de Luminy, 13288 Marseille, France

An anaerobic, thermophilic, filamentous (0.45 × >100 μm) bacterium, designated D1-25-10-4^T, was isolated from a deep hot aquifer in France. Cells were non-motile and Gram-negative. Growth was observed at 43–65 °C (optimum 55 °C), at pH 6.8–7.8 (optimum pH 7.0) and with 0–5 g NaCl l^{-1} (optimum 0 g NaCl l^{-1}). Strain D1-25-10-4^T was a chemo-organotroph and fermented ribose, maltose, glucose, galactose, arabinose, fructose, mannose, sucrose, raffinose, xylose, glycerol, fumarate, peptone, starch and xylan. Yeast extract was required for growth. Sulfate, thiosulfate, sulfite, elemental sulfur, nitrate, nitrite and fumarate were not used as terminal electron acceptors. The G+C content of the DNA was 61.9 mol%. The major cellular fatty acids of strain D1-25-10-4^T were $C_{17:0}$, $C_{18:0}$, $C_{16:0}$ and iso-$C_{17:0}$. The closest phylogenetic relative of strain D1-25-10-4^T was *Caldilinea aerophila* STL-6-O1^T (97.9 % 16S rRNA gene sequence similarity). DNA–DNA relatedness between strain D1-25-10-4^T and *Caldilinea aerophila* DSM 14535^T was 8.7 ± 1 %. On the basis of phylogenetic, genotypic and phenotypic characteristics, strain D1-25-10-4^T represents a novel species within the genus *Caldilinea*, class *Caldilineae*, phylum *Chloroflexi*, for which the name *Caldilinea tarbellica* sp. nov. is proposed. The type strain is D1-25-10-4^T (=DSM 22659^T =JCM 16120^T).

The phylum *Chloroflexi* is a deeply branched bacterial lineage that is known from numerous environmental 16S rRNA gene sequences but contains few cultured representatives. So far, the phylum comprises six classes: *Anaerolineae*, *Caldilineae*, '*Chloroflexi*', '*Dehalococcoidetes*', *Ktedonobacteria* and *Thermomicrobia* (Castenholz, 2001; Cavaletti *et al.*, 2006; Hugenholtz & Stackebrandt, 2004; Moe *et al.*, 2009; Yamada *et al.*, 2006, 2007). Cultured members of the class '*Chloroflexi*' may be able to grow phototrophically under anaerobic (e.g. *Oscillochloris trichoides* and *Chloroflexus aggregans*; Hanada *et al.*, 1995a; Keppen *et al.*, 2000) or aerobic (e.g. *Chloroflexus aurantiacus*, *Roseiflexus castenholzii* and *Heliothrix oregonensis*; Hanada *et al.*, 1995b, 2002; Pierson *et al.*, 1985) conditions. They may be mesophiles (e.g. *Oscillochloris trichoides*; Keppen *et al.*, 2000) or thermophiles (e.g. *Roseiflexus castenholzii*; Hanada *et al.*, 2002). In contrast, members of the five other classes of the phylum *Chloroflexi* are considered mesophilic (e.g. *Longilinea arvoryzae*; Yamada *et al.*, 2007) or thermophilic (e.g. *Dehalogenimonas lykanthroporepellens*; Moe *et al.*, 2009) chemo-organotrophic anaerobes, with the exceptions of *Caldilinea aerophila* (class *Caldilineae*, family *Caldilineaceae*), a facultatively anaerobic thermophile isolated from a hot spring sulfur-turf (Sekiguchi *et al.*, 2003), and the aerobic, thermophilic members of the class *Thermomicrobia* (Botero *et al.*, 2004; Demharter *et al.*, 1989; Jackson *et al.*, 1973). Most cultivated thermophilic members of the phylum *Chloroflexi* have been isolated from bacterial mats of hot springs (Botero *et al.*, 2004; Garrity & Holt, 2001a, b, Hanada *et al.*, 2002; Jackson *et al.*, 1973; Sekiguchi *et al.*, 2003) and from thermophilically treated sewage sludge (Demharter *et al.*, 1989; Yamada *et al.*, 2007).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain D1-25-10-4^T is HM134893.

We have recently undertaken studies of the microbial diversity in two deep hot aquifers located in south-west France (Dax, St Paul-lès-Dax). Both aquifers are of a meteoric origin (Le Fanic, 2005) and have temperatures up to 68 °C. Samples were collected under anaerobic conditions from the head pump of Saint-Christophe spring at a depth of 149 m. At the time of sampling, the water was 60 °C and pH 7.3 and had a conductivity (at 25 °C) of 1458 μS cm^{-1}. Anions in the water were measured by ion chromatography and cations by inductively coupled plasma-optical emission spectrometry (ICP-OES). The water contained (l^{-1}) 147 mg chloride, 381 mg sulfate, 158.3 mg hydrogencarbonate, 1.6 mg fluoride, 125 mg sodium, 123 mg calcium, 31 mg magnesium, 26 mg potassium, 0.07 mg Fe(II) and 0.15 mg ammonium. Samples were stored in the laboratory at room temperature. Enrichment was performed in Hungate tubes (Hungate, 1969) containing 500 ml aquifer water and 5 mM acetate, 1 g NH_4Cl l^{-1}, 20 mM glucose, 0.1 g yeast extract l^{-1} and 0.04 % (w/v) $Na_2S.9H_2O$ (added from sterile stock solutions) at 60 °C under a stream of O_2-free N_2 gas. Positive growth was obtained after 6 months at 60 °C. Subsequent enrichment was performed in Hungate tubes containing 4.5 ml aquifer water under the same growth conditions. Cultures were purified by tenfold serial dilution in roll tubes with medium supplemented with 2.5 % agar. Round, white colonies (about 1 mm in diameter) developed after 2 months. Single colonies were subcultivated by the process of serial dilution until cultures were deemed to be axenic. Several filamentous strains similar in phylogeny, morphology and metabolic profile from glucose fermentation were isolated. One strain, designated D1-25-10-4^T, was used for further characterization. *Caldilinea aerophila* DSM 14535^T was obtained from the DSMZ (Braunschweig, Germany) for comparison, notably under anaerobic conditions.

For routine cultivation, the following basal medium was used (per litre distilled water): 0.3 g KH_2PO_4, 0.3 g K_2HPO_4, 1 g NH_4Cl, 1 g NaCl, 0.1 g KCl, 0.1 g $CaCl_2.2H_2O$, 0.5 g $MgCl_2.6H_2O$, 0.5 g cysteine hydrochloride, 1 g yeast extract, 1 g biotrypticase (Difco), 10 ml trace element solution (Balch *et al.*, 1979) and 1 ml 0.1 % resazurin solution. The pH was adjusted to pH 7.2 at 25 °C with 10 M KOH solution, boiled under a stream of O_2-free N_2 gas and cooled to room temperature. Aliquots (4.5 ml) were dispensed into Hungate tubes, degassed under N_2/CO_2 (80 : 20, v/v) and sterilized by autoclaving at 120 °C for 20 min. Prior to inoculation, 0.1 ml 2 % (w/v) $Na_2S.9H_2O$, 0.1 ml 10 % (w/v) $NaHCO_3$ and 20 mM glucose from sterile stock solutions were injected into the tubes.

Morphological characteristics and purity were observed by phase-contrast microscopy (Optiphot; Nikon). For transmission electron microscopy, cell preparations were negatively stained with sodium phosphotungstate as described previously (Fardeau *et al.*, 1997). The presence of spores was checked by microscopic observation. Pasteurization tests were performed at 80, 90 and 100 °C for 10 and 20 min.

All experiments were duplicated and growth was tested by subculturing the isolate at least once using glucose (20 mM) as the electron donor. To determine growth at pH 5.0–9.3, the pH of the medium was adjusted at 25 °C with sterile anaerobic stock solutions of 0.1 M HCl, 10 % (w/v) $NaHCO_3$ or 8 % (w/v) Na_2CO_3. To test for NaCl requirement, NaCl was weighed directly into the tubes to give final concentrations from 0 to 50 g NaCl l^{-1}. The temperature range for growth was tested between 37 and 80 °C. The substrates tested were ribose, glucose, galactose, maltose, arabinose, fructose, raffinose, mannose, sucrose, xylose, lactate, malate, acetate, succinate, fumarate, pyruvate, peptone, ethanol, glycerol (all at 20 mM), formate (80 mM), xylan (mixture of birch wood and oat spelt; 2 g l^{-1}), starch (10 g l^{-1}), Casamino acids (2 g l^{-1}) and H_2/CO_2 (80 : 20, v/v, at 2 bars in the presence or absence of 2 mM acetate as the carbon source). The electron acceptors tested were sodium sulfate (20 mM), sodium thiosulfate (20 mM), sodium sulfite (2 mM), elemental sulfur (10 g l^{-1}), sodium nitrite (2 mM), sodium nitrate (10 mM) and sodium fumarate (20 mM), in the presence of 20 mM glucose as the energy source. The effect of O_2 on growth was determined in Hungate tubes containing anaerobic basal medium supplemented with glucose. Tubes were inoculated and various amounts of sterile air were added to the gas phase to obtain final oxygen concentrations of 0.5, 1, 1.5, 2, 5, 10 and 21 %. Cultures were incubated at 60 °C at 140 r.p.m. Bacterial growth was monitored by measuring the increase in optical density at 580 nm by inserting Hungate tubes into the cuvette holder of a spectrophotometer (Cary 50; Varian). H_2S production was determined photometrically as colloidal CuS by the method of Cord-Ruwisch (1985). End products of metabolism after 2 weeks at 60 °C were measured by HPLC (Spectra Series 100 model; Thermo Separation Products) with an Aminex HPX 87 × 300 mm long, 7.8 mm i.d. column (Bio-Rad) connected to a differential refractometer (RID-6A; Shimadzu). Analysis was performed using a CR-6A Shimadzu integrator. The mobile phase was 0.01 M H_2SO_4 and had a flow rate of 0.5 ml min^{-1} and the column temperature was 35 °C. The volume of the injection loop was 20 ml. H_2 and CO_2 were measured using a gas chromatograph (GC-8A; Shimadzu) equipped with a carbosphere column maintained at 150 °C. The injector and the catharometer were maintained at 200 °C (Fardeau *et al.*, 1993). Xylanolytic activity was measured as described previously (Fardeau *et al.*, 1997). Susceptibility to ampicillin, chloramphenicol and penicillin was tested at 50, 100, 150 and 200 μg ml^{-1}.

The composition of fatty acids, G+C content and DNA–DNA relatedness were determined by the DSMZ (Braunschweig, Germany). Fatty acid methyl esters were extracted from fresh biomass of strain D1-25-10-4^T and *Caldilinea aerophila* DSM 14535^T grown in the basal medium and identified following the procedure recommended by the

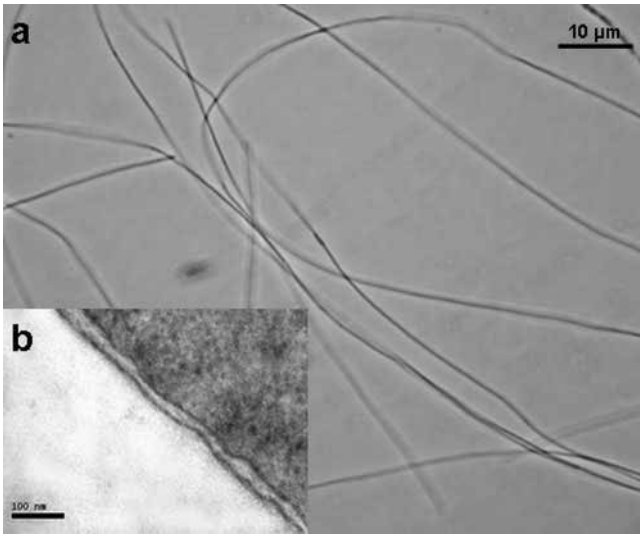

Fig. 1. (a) Phase-contrast micrograph of filamentous forms of strain D1-25-10-4^T. Bar, 10 µm. (b) Transmission electron micrograph of a thin section of a cell of strain D1-25-10-4^T grown on glucose, showing the two electron-dense layers of the cell wall. Bar, 100 nm.

Microbial Identification system (version 4.0, MIS operating manual March 2001; MIDI). The MIS system was used to compare the fatty acid methyl ester profiles of strain D1-25-10-4^T and the reference strain with fatty acid patterns stored in MIDI fatty acid database. DNA was purified by chromatography on hydroxyapatite by the procedure of Cashion *et al.* (1977). G+C content was determined using HPLC as described by Mesbah *et al.* (1989). Non-methylated lambda DNA (Sigma) was used as the standard. DNA–DNA hybridization analysis was performed as described by De Ley *et al.* (1970) with modifications as reported by Escara & Hutton (1980) and Huß *et al.* (1983) using a Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6×6 multicell changer and a temperature controller with an *in situ* temperature probe.

For 16S rRNA gene sequence analysis, DNA was extracted using the Wizard genomic DNA purification kit (Promega), according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the universal primers 27F (5′-AGAGTTTGATCCTGGC-TCAG-3′; *Escherichia coli* positions 8–27) and 1492R (5′-TACGGTTACCTTGTTACGAC-3′; *E. coli* positions 1492–1511) (Lane, 1991). Direct sequencing of PCR products was performed by GATC Biotech. Reference sequences were obtained from the Ribosomal Database Project II (Maidak *et al.*, 2001), EMBL and GenBank

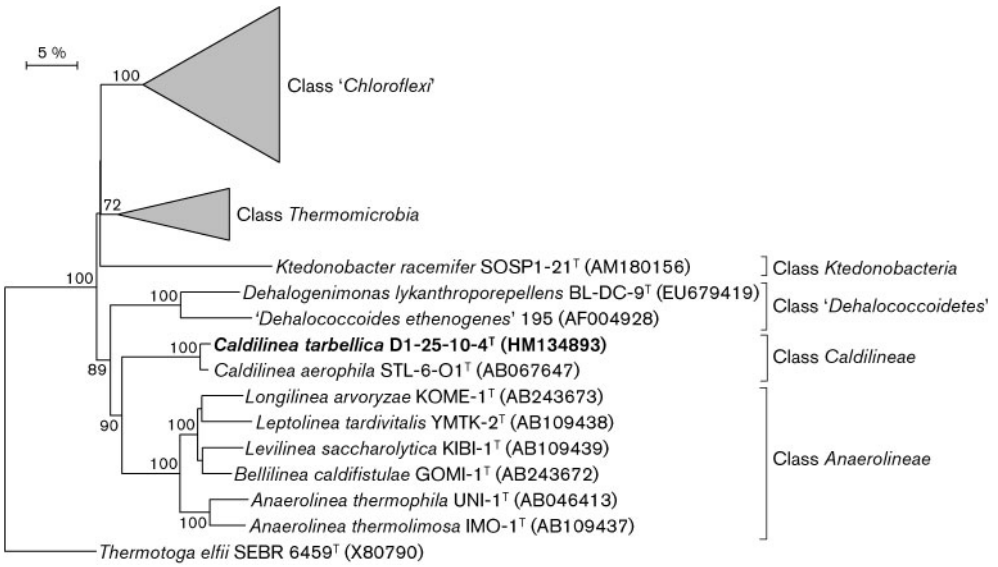

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain D1-25-10-4^T within the phylum *Chloroflexi*. The triangle representing the class '*Chloroflexi*' represents the sequences of *Chloroflexus aurantiacus* J-10-fl^T (GenBank accession no. D38365), *Chloroflexus aggregans* MD-66^T (D32255), *Oscillochloris trichoides* DG-6^T (AF093427), *Chloronema giganteum* Gnsb-1^T (AF345825), *Roseiflexus castenholzii* HLO8^T (AB041226), *Herpetosiphon geysericola* ATCC 23076^T (AF039293) and *Herpetosiphon aurantiacus* ATCC 23779^T (CP000875), and that representing the class *Thermomicrobia* represents the sequences of '*Thermobaculum terrenum*' YNP1 (AF391972), *Thermomicrobium roseum* DSM 5159^T (M34115) and *Sphaerobacter thermophilus* DSM 20745^T (AJ420142). Bootstrap values (>70 %) based on 1000 resamplings are shown at branch nodes. *Thermotoga elfii* SEBR 6459^T was used as an outgroup. Bar, 5 substitutions per 100 nucleotides.

databases (Benson *et al.*, 1999). The nucleotide sequence of the 16S rRNA gene was aligned manually with reference sequences of various members of the phylum *Chloroflexi* using BioEdit (Hall, 1999). Nucleotide ambiguities were omitted and evolutionary distances were calculated using the Jukes and Cantor option (Jukes & Cantor, 1969) in TREECON (Van de Peer & De Wachter, 1997). Phylogenetic trees were constructed using the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood and maximum-parsimony methods. Confidence in tree topologies was determined by bootstrap analysis based on 1000 resamplings (Felsenstein, 1985).

Cells of strain D1-25-10-4^T were non-motile, Gram-negative, anaerobic thermophilic filaments. Their growth was inhibited after treatment at 100 °C for 20 min. They did not use O_2 as an electron acceptor, but tolerated up to 1.5 % O_2 in the gas phase. Cells were approximately 0.45 μm in diameter and more than 100 μm long (Fig. 1a). Electron microscopy revealed a multilayered cell wall outside the cytoplasmic membrane. The cell wall consisted of two electron-dense layers and one electron-transparent layer (Fig. 1b). The isolate grew at 43–65 °C (optimum 55 °C), at pH 6.8–7.8 (optimum pH 7.0) and with 0–5 g NaCl l^{-1} (optimum 0 g NaCl l^{-1}). Strain D1-25-10-4^T grew with 200 μg penicillin ml^{-1}, but not with 50 μg ampicillin or 50 μg chloramphenicol ml^{-1}. Strain D1-25-10-4^T was a chemo-organotroph and used ribose, maltose, glucose, galactose, arabinose, fructose, mannose, sucrose, raffinose, xylose, glycerol, fumarate, peptone, starch and xylan. Yeast extract (0.1 %) was required for growth. Growth was improved by biotrypticase (0.1 %). Succinate and lactate were the main end products of glucose fermentation and minor end products were acetate, CO_2 and H_2 (trace). Fumarate was converted to malate. The following compounds were not used as energy sources: lactate, malate, acetate, succinate, pyruvate, formate (with or without acetate as carbon source), ethanol, Casamino acids and H_2 (with or without acetate as carbon source). Xylanase activity was shown to be extracellular. The minimum doubling time using glucose as energy source was 21 h at 55 °C and pH 7.2 with 1 g NaCl l^{-1}.

The G+C content of the DNA was 61.9 mol%. Phylogenetic analysis of the almost-complete sequence (1400 bp) of the 16S rRNA gene of strain D1-25-10-4^T showed its affiliation to the phylum *Chloroflexi* (Fig. 2). The topology of the neighbour-joining tree was also supported by trees generated with the maximum-parsimony and maximum-likelihood algorithms. The closest phylogenetic neighbour of strain D1-25-10-4^T was *Caldilinea aerophila* STL-6-O1^T (97.9 % 16S rRNA gene sequence similarity). DNA–DNA relatedness between strain D1-25-10-4^T and *Caldilinea aerophila* DSM 14535^T was 8.7±1 %, which suggested that strain D1-25-10-4^T represented a novel species.

Caldilinea aerophila is considered to be a facultative anaerobe and has been previously characterized only under aerobic conditions (Sekiguchi *et al.*, 2003). However, despite repeated trials, strain D1-25-10-4^T never grew aerobically in our basal medium or in the original medium described for the cultivation of *Caldilinea aerophila* (Sekiguchi *et al.*, 2003). Therefore, to differentiate strain D1-25-10-4^T phenotypically from its closest phylogenetic neighbour, the metabolic properties of both the isolate and the reference strain were studied under anaerobic conditions using our basal medium. The results of the phenotypic analysis are presented in Table 1. Strain

Table 1. Characteristics that differentiate strain D1-25-10-4^T from *Caldilinea aerophila* DSM 14535^T

Data were obtained in this study under anaerobic conditions unless otherwise stated. Both strains grew with 0–5 g NaCl l^{-1} (optimum 0 g NaCl l^{-1}) and used ribose, maltose, glucose, galactose, arabinose, fructose, mannose, raffinose, fumarate, peptone and xylan. Neither strain was motile* or used lactate, acetate, succinate, pyruvate, formate, H_2, ethanol or Casamino acids. ++, Very good growth (OD_{580}>0.25); +, growth; ±, poor growth (OD_{580}<0.15); −, no growth.

Characteristic	Strain D1-25-10-4^T	*C. aerophila* DSM 14535^T
DNA G+C content (mol%)	61.9	59.0*
Temperature for growth (°C)		
Range	43–65	37–65*
Optimum	55	55*
pH for growth		
Range	6.8–7.8	7.0–9.0*
Optimum	7.0	7.5–8.0*
Electron donors		
Sucrose	±	++
Xylose	±	++
Malate	−	±
Glycerol	+	−
Starch	±	++
Yeast extract	±	+

*Data for *Caldilinea aerophila* STL-6-O1^T determined under aerobic conditions by Sekiguchi *et al.* (2003).

Table 2. Major cellular fatty acids of strain D1-25-10-4^T and *Caldilinea aerophila* DSM 14535^T

Values are percentages of total fatty acids and were obtained in this study. Fatty acids representing <3 % of the total are not shown.

Fatty acid	Strain D1-25-10-4^T	*C. aerophila* DSM 14535^T
$C_{16:0}$	17.12	13.28
$C_{17:0}$	30.77	11.66
iso-$C_{17:0}$	10.29	19.17
$C_{18:0}$	23.38	35.42

D1-25-10-4^T differed from *Caldilinea aerophila* DSM 14535^T by not using malate and by fermenting glycerol. Both strains produced succinate, acetate, CO_2 and traces of H_2 as end products from glucose metabolism, but strain D1-25-10-4^T also produced lactate. Neither strain used sulfate, thiosulfate, sulfite, elemental sulfur, nitrate, nitrite or fumarate as terminal electron acceptors.

Comparison of the major fatty acids of the isolate and the reference strain supported the finding that strain D1-25-10-4^T differed from *Caldilinea aerophila* DSM 14535^T (Table 2). For example, the dominant fatty acid of strain D1-25-10-4^T was $C_{17:0}$ (30.77 %), whereas the dominant fatty acid of *Caldilinea aerophila* DSM 14535^T was $C_{18:0}$ (35.42 %). In addition, the obligately anaerobic character of strain D1-25-10-4^T and the facultatively anaerobic character of *Caldilinea aerophila* DSM 14535^T differentiated these two bacteria.

On the basis of several phenotypic, genotypic, phylogenetic and chemotaxonomic features that distinguish strain D1-25-10-4^T from its closest phylogenetic neighbour, strain D1-25-10-4^T should be classified in a novel species of the genus *Caldilinea*. The name *Caldilinea tarbellica* sp. nov. is proposed.

Description of *Caldilinea tarbellica* sp. nov.

Caldilinea tarbellica (tar.bel'li.ca. L. fem. adj. *tarbellica* Tarbellian, here intended to mean belonging to Aquae Tarbellicae, the Roman name of Dax, referring to the origin of the type strain).

Cells are anaerobic, Gram-negative, non-motile, non-sporulating filaments, approximately 0.45 μm in diameter and over 100 μm long. Grows at 43–65 °C (optimum 55 °C). NaCl is not required for growth. The optimum pH for growth is pH 7.0. Uses ribose, maltose, glucose, galactose, arabinose, fructose, mannose, sucrose, raffinose, xylose, glycerol, fumarate, peptone, starch and xylan, but not lactate, acetate, malate, succinate, pyruvate, formate, ethanol, Casamino acids or H_2. Glucose is fermented into succinate, lactate, acetate, CO_2 and traces of H_2. The major cellular fatty acids are $C_{17:0}$, $C_{18:0}$, $C_{16:0}$ and iso-$C_{17:0}$. The DNA G+C content of the type strain is 61.9 mol%.

The type strain is D1-25-10-4^T (=DSM 22659^T =JCM 16120^T), isolated from a deep hot aquifer in south-west France (Dax, Aquitaine Basin).

Acknowledgements

This work was supported by AFRETH and the Institut du Thermalisme – Université Victor Segalen Bordeaux 2. We thank Dr F. Hamaide for supporting this research and Sandra Biasutti and Dr Joël Lagière for technical assistance. We also give many thanks to M. G. Ponteins, director of the Thermadour group, for giving access to the hot spring, Dr J. P. Euzéby for checking the etymology of the species name and Dr P. Roger for improving the manuscript.

References

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.

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

Botero, L. M., Brown, K. B., Brumefield, S., Burr, M., Castenholz, R. W., Young, M. & McDermott, T. R. (2004). *Thermobaculum terrenum* gen. nov., sp. nov.: a non-phototrophic gram-positive thermophile representing an environmental clone group related to the *Chloroflexi* (green non-sulfur bacteria) and *Thermomicrobia*. *Arch Microbiol* **181**, 269–277.

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.

Castenholz, C. W. (2001). Class I. '*Chloroflexi*'. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, p. 427. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Cavaletti, L., Monciardini, P., Bamonte, R., Schumann, P., Rohde, M., Sosio, M. & Donadio, S. (2006). New lineage of filamentous, spore-forming, gram-positive bacteria from soil. *Appl Environ Microbiol* **72**, 4360–4369.

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

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Demharter, W., Hensel, R., Smida, J. & Stackebrandt, E. (1989). *Sphaerobacter thermophilus* gen. nov., sp. nov. A deeply rooting member of the *Actinomycetes* subdivision isolated from thermophilically treated sewage sludge. *Syst Appl Microbiol* **11**, 261–266.

Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.

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.

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 Bacteriol* **47**, 1013–1019.

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

Garrity, G. M. & Holt, J. G. (2001a). Phylum BVI. *Chloroflexi* phy. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, p. 427. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Garrity, G. M. & Holt, J. G. (2001b). Phylum BVII. *Thermomicrobia* phy. nov. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, p. 447. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

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.

Hanada, S., Hiraishi, A., Shimada, K. & Matsuura, K. (1995a). *Chloroflexus aggregans* sp. nov., a filamentous phototrophic bacterium which forms dense cell aggregates by active gliding movement. *Int J Syst Bacteriol* **45**, 676–681.

Hanada, S., Hiraishi, A., Shimada, K. & Matsuura, K. (1995b). Isolation of *Chloroflexus aurantiacus* and related thermophilic phototrophic bacteria from Japanese hot springs using an improved isolation procedure. *J Gen Appl Microbiol* **41**, 119–130.

Hanada, S., Takaichi, S., Matsuura, K. & Nakamura, K. (2002). *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes. *Int J Syst Evol Microbiol* **52**, 187–193.

Hugenholtz, P. & Stackebrandt, E. (2004). Reclassification of *Sphaerobacter thermophilus* from the subclass *Sphaerobacteridae* in the phylum *Actinobacteria* to the class *Thermomicrobia* (emended description) in the phylum *Chloroflexi* (emended description). *Int J Syst Evol Microbiol* **54**, 2049–2051.

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

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Jackson, T. J., Ramaley, R. F. & Meinschein, W. G. (1973). *Thermomicrobium*, a new genus of extremely thermophilic bacteria. *Int J Syst Bacteriol* **23**, 28–36.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Keppen, O. I., Tourova, T. P., Kuznetsov, B. B., Ivanovsky, R. N. & Gorlenko, V. M. (2000). Proposal of *Oscillochloridaceae* fam. nov. on the basis of a phylogenetic analysis of the filamentous anoxygenic phototrophic bacteria, and emended description of *Oscillochloris* and *Oscillochloris trichoides* in comparison with further new isolates. *Int J Syst Evol Microbiol* **50**, 1529–1537.

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.

Le Fanic, R. (2005). *Hydrogéologie d'un système thermal et modélisation couplée hydrodynamique, thermique en vue de la gestion de la ressource*. PhD thesis, Université Bordeaux 3, France (in French).

Maidak, B. L., Cole, J. R., Lilburn, T. G., Parker, C. T., Jr, Saxman, P. R., Farris, R. J., Garrity, G. M., Olsen, G. J., Schmidt, T. M. & Tiedje, J. M. (2001). The RDP-II (Ribosomal Database Project). *Nucleic Acids Res* **29**, 173–174.

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.

Moe, W. M., Yan, J., Nobre, M. F., da Costa, M. S. & Rainey, F. A. (2009). *Dehalogenimonas lykanthroporepellens* gen. nov., sp. nov., a reductively dehalogenating bacterium isolated from chlorinated solvent-contaminated groundwater. *Int J Syst Evol Microbiol* **59**, 2692–2697.

Pierson, B. K., Giovannoni, S. J., Stahl, D. A. & Castenholz, R. W. (1985). *Heliothrix oregonensis*, gen. nov., sp. nov., a phototrophic filamentous gliding bacterium containing bacteriochlorophyll a. *Arch Microbiol* **142**, 164–167.

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

Sekiguchi, Y., Yamada, T., Hanada, S., Ohashi, A., Harada, H. & Kamagata, Y. (2003). *Anaerolinea thermophila* gen. nov., sp. nov. and *Caldilinea aerophila* gen. nov., sp. nov., novel filamentous thermophiles that represent a previously uncultured lineage of the domain *Bacteria* at the subphylum level. *Int J Syst Evol Microbiol* **53**, 1843–1851.

Van de Peer, Y. & De Wachter, R. (1997). Construction of evolutionary distance trees with TREECON for Windows: accounting for variation in nucleotide substitution rate among sites. *Comput Appl Biosci* **13**, 227–230.

Yamada, T., Sekiguchi, Y., Hanada, S., Imachi, H., Ohashi, A., Harada, H. & Kamagata, Y. (2006). *Anaerolinea thermolimosa* sp. nov., *Levilinea saccharolytica* gen. nov., sp. nov. and *Leptolinea tardivitalis* gen. nov., sp. nov., novel filamentous anaerobes, and description of the new classes *Anaerolineae* classis nov. and *Caldilineae* classis nov. in the bacterial phylum *Chloroflexi*. *Int J Syst Evol Microbiol* **56**, 1331–1340.

Yamada, T., Imachi, H., Ohashi, A., Harada, H., Hanada, S., Kamagata, Y. & Sekiguchi, Y. (2007). *Bellilinea caldifistulae* gen. nov., sp. nov. and *Longilinea arvoryzae* gen. nov., sp. nov., strictly anaerobic, filamentous bacteria of the phylum *Chloroflexi* isolated from methanogenic propionate-degrading consortia. *Int J Syst Evol Microbiol* **57**, 2299–2306.