

Caminibacter hydrogeniphilus gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing bacterium isolated from an East Pacific Rise hydrothermal vent

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A novel thermophilic, anaerobic, hydrogen-oxidizing bacterium, designated strain AM1116^T, was isolated from an East Pacific Rise hydrothermal vent sample. The cells were rod-shaped (1.0–1.5 × 0.5 µm), motile with polar flagella. They grew at temperatures between 50 and 70 °C (optimum 60 °C; doubling time approximately 1.5 h), at between pH 5.0 and 7.5 (optimum around pH 5.5–6.0) and in between 10 and 40 g NaCl l⁻¹ (optimum 20–25 g l⁻¹). Cells grew chemolithoautotrophically in a H₂/CO₂ atmosphere (80:20; 200 kPa). Poor heterotrophic growth was observed on complex organic substrates. Elemental sulphur and nitrate served as electron acceptors, respectively yielding hydrogen sulphide and ammonia (doubling times were equal with the two electron acceptors). In contrast, when cystine was used as electron acceptor, growth was poor. The G+C content of the genomic DNA was 29±1 mol%. Phylogenetic analyses of the 16S rRNA gene located the strain within the ϵ -Proteobacteria, in the bacterial domain. On the basis of 16S rDNA sequence comparisons, physiological and biochemical characteristics, it is proposed that the isolate should be described as the type species of a new genus, *Caminibacter* gen. nov., as *Caminibacter hydrogeniphilus* sp. nov. The type strain is strain AM1116^T (= DSM 14510^T = CIP 107140^T).

Keywords: deep-sea hydrothermal vent, thermophile, lithotroph, ϵ -Proteobacteria, *Caminibacter hydrogeniphilus*

INTRODUCTION

The subclass ϵ -Proteobacteria comprises a complex group of Gram-negative, microaerophilic and/or anaerobic, chemoorganoheterotrophic or chemolithotrophic bacteria that are found in a variety of habitats. Many members of this group are eukaryotic pathogens. It currently contains two major lineages. The first lineage encompasses the genera *Arcobacter*, *Sulfurospirillum*, *Campylobacter*, *Thiovulum*, *Helicobacter* and *Wolinella*. In addition, some members of this lineage

are found to be the dominant ectosymbionts of some hydrothermal vent metazoans. The single phylotype recovered from nucleic acids extracted from epibionts of the Atlantic hydrothermal shrimp *Rimicaris exoculata* was found to belong to the ϵ -Proteobacteria and also to be the major component of the free-living substrate community at this site (Polz & Cavanaugh, 1995). The filamentous ectosymbionts of the annelid polychaete *Alvinella pompejana* (Haddad *et al.*, 1995; Cary *et al.*, 1997), thriving at East Pacific Rise hydrothermal vents, were also found to belong to this lineage. These ectosymbionts were thought to contribute to protection against sulphide toxicity and/or to the nutrition of their host (Alayse-Danet *et al.*, 1987; Desbruyères *et al.*, 1998). Many attempts to culture them have been made in our laboratory (unpublished results) and in other laboratories (Prieur

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& Jeanthon, 1987; Prieur *et al.*, 1990; Jeanthon & Prieur, 1990; Campbell *et al.*, 2001), under a wide range of temperatures in combination with different electron donors, electron acceptors and carbon sources, but, to date, none of them has been successful.

The second lineage contains environmental clone sequences from different hydrothermal communities that have been investigated by molecular phylogenetic approaches. Members of the *ε-Proteobacteria* have been identified from both deep-sea Atlantic and Pacific vents (Polz & Cavanaugh, 1995; Moyer *et al.*, 1995; Reysenbach *et al.*, 2000; Longnecker & Reysenbach, 2001; Campbell *et al.*, 2001). In all the deep-sea hydrothermal vent microbial communities studied, *ε-Proteobacteria* phylotypes were found to be dominant, accounting for 40–98% of the bacterial clone sequences (Moyer *et al.*, 1995; Reysenbach *et al.*, 2000; Longnecker & Reysenbach, 2001). Analyses of their numerical importance, using fluorescent *in situ* hybridization or 16S rRNA-based quantitative oligonucleotide hybridization techniques, have demonstrated that these *ε*-proteobacteria were abundant, and even dominant, in the communities studied (Polz & Cavanaugh, 1995; Longnecker & Reysenbach, 2001). Their metabolism, their potential impact on the biogeochemistry and their place in the food chain of this ecosystem have remained undefined because, until recently (Campbell *et al.*, 2001), all attempts to culture them have failed.

In 1999, hydrothermal samples were collected from a deep-sea vent field located on the East Pacific Rise (13° N). In this paper, we describe the isolation and the characterization of a thermophilic member of the *ε-Proteobacteria* belonging to the second lineage.

METHODS

Collection of samples. In 1999, a deep-sea vent field located on the East Pacific Rise [Elsa (HOT3), 103° 56' W, 12° 48' N] was explored during the *AMISTAD (Advanced Microbiological Studies of Thermophiles: Adaptations and Diversity)* oceanographic cruise. Entire young tube samples of the hydrothermal vent polychaete *Alvinella pompejana*, which inhabits the surface of active sulphide structures ('chimneys', 'smokers'), attached to small fragments of chimney rock, were collected by the man-operated *Nautilite* submersible. The temperature ranged from 10 to 100 °C and the pressure was 26 MPa at the hydrothermal sampling site and the temperature recorded in the *Alvinella pompejana* colony was approximately 45–49 °C with a pH of 5.8. After collection, the samples were transferred immediately into an anaerobic chamber and then, after removal of the tube's inner contents, into 50 ml serum vials filled with a sterile solution of 3% (v/v) sea salts (Sigma). They were stored at 4 °C until enrichment in the laboratory.

Culture medium and conditions. The isolate was enriched and grown on KA22 medium containing (l⁻¹): 30 g sea salts (Sigma), 2.54 g Mg(NO₃)₂·6H₂O (Merck), 1.95 g MES buffer (Sigma), 12 g elemental sulphur (Prolabo), 0.3 g KH₂PO₄ (Sigma), 0.5 ml vitamin mixture (Balch *et al.*, 1979), 10 ml trace element solution (Balch *et al.*, 1979) and

1 mg resazurin (Sigma). The pH of the medium was adjusted to 6.0 at room temperature before autoclaving and the medium was reduced with 0.5 g sodium sulphide l⁻¹ before inoculation. A mixture of H₂/CO₂ (80:20; 200 kPa) was used as the gas phase. Unless indicate otherwise, cultures were incubated at 60 °C.

Enrichment and purification procedures. Cultures were enriched anaerobically in KA22 medium inoculated with solid fragments of young *Alvinella pompejana* tubes, in 50 ml serum vials (Balch & Wolfe, 1976). They were incubated at 60 °C for 20 h under an atmosphere of H₂/CO₂ (80:20; 200 kPa). Cells were visualized by microscope observation. After enrichment, positive cultures were subcultured and then purified by use of the dilution-to-extinction technique (Baross, 1995). Purity of the isolate was confirmed by microscope observations and by cloning and sequencing of ten independent 16S rDNA clones.

Observation of the culture and quantification. Cells were observed under a light microscope (model BH2; Olympus) equipped with a phase-contrast oil immersion objective (×100 magnification). Cells were quantified by direct cell counting using a Thoma chamber (depth 0.02 mm). Alternatively, cells were fixed with 1% (v/v) glutaraldehyde for 45 min at room temperature and stored at –20 °C before being counted in a Thoma chamber.

Morphology. The cells were Gram stained by use of the Bacto 3-step Gram stain Set-S (Difco). SpotTest flagella stain (Difco) was used for flagella detection. For scanning electron microscopy, cells were fixed with 10% formaldehyde (v/v) for 1 h and then harvested by centrifugation for 20 min at 1000 g. The pellets were resuspended in 23 g NaCl l⁻¹, displayed on filters (0.22 µm pore size, Nucleopore) and dried overnight at room temperature. Samples were then coated with gold (SCD040; Balzers) and examined with a scanning electron microscope (XL 30 LaB6; Philips).

Determination of growth parameters. To determine the optimum temperature, pH and NaCl for growth, cells were grown in Hungate tubes (27 ml; Bellco) containing 5 ml KA22 medium and H₂/CO₂ at a pressure of 200 kPa in the headspace. Experiments were performed in thermostatic aluminium heating blocks (Bioblock) monitored with temperature probes placed in control tubes. To determine the effect of pH on growth, KA22 medium was modified with the following buffers (each at 10 mM; Sigma): for pH 3 and 4, no buffer; for pH 5, 5.5 and 6, MES; for pH 6.5 and 7, PIPES; for pH 7.5 and 8, HEPES; for pH 8.5 and 9, AMPSO. Sodium sulphide was added in the anaerobic chamber and, if necessary, the pH was adjusted with 0.1 M HCl or 0.1 M NaOH. To determine the effect of salinity on growth, KA22 medium was prepared without sea salts and with different concentrations of NaCl. For this experiment, the following salts were added to the medium (l⁻¹): 1.0 g NH₄Cl, 0.2 g MgCl₂·6H₂O, 0.1 g CaCl₂·2H₂O, 0.1 g KCl and 0.3 g K₂HPO₄. Cells were then incubated at the optimum temperature and pH for growth. The effects of temperature, pH and salinity were determined by measuring growth rates calculated by use of linear regression analyses from four to seven points along the logarithmic portions of the growth curves. All growth experiments were carried out in triplicate.

Determination of growth requirements. The ability of isolate AM1116^T to use various electron donors was investigated. Formate (20 mM), acetate (20 mM), 0.5% methanol (v/v) or 2 g yeast extract l⁻¹ were added to the KA22 medium with a N₂/CO₂ gas phase (80:20, 200 kPa).

The ability of the novel isolate to grow in the presence of different electron acceptors was tested on KA22 medium prepared without sulphur in which $\text{Mg}(\text{NO}_3)_2$ was replaced with $1 \text{ g NH}_4\text{Cl l}^{-1}$. Elemental sulphur and L-cystine were tested at 12 g l^{-1} , polysulphides at 10 mM (Blumentals *et al.*, 1990) and thiosulphate, sulphate, nitrate and nitrite at 20 mM . The headspace gas was H_2/CO_2 (80:20; 200 kPa). Microaerobic media, containing a gas phase of H_2/CO_2 (200 kPa) and low concentrations of oxygen [from approximately 0.05 to 0.5% (v/v) O_2], were prepared without resazurin and sodium sulphide.

The ability of strain AM1116^T to utilize different carbon sources was investigated by adding the following compounds to the KA22 medium: formate (20 mM), acetate (20 mM), lactate (0.5% v/v), methanol (0.5% v/v), yeast extract (2 g l⁻¹), tryptone (2 g l⁻¹), brain/heart infusion (2 g l⁻¹), peptone (2 g l⁻¹), gelatin (2 g l⁻¹), glycogen (2 g l⁻¹), D-(+)-glucose (2 g l⁻¹), D-(-)-fructose (2 g l⁻¹), D-(+)-galactose (2 g l⁻¹), maltose (2 g l⁻¹), starch (2 g l⁻¹), chitin (2 g l⁻¹), dextran (2 g l⁻¹), xylan (2 g l⁻¹), cellulose (2 g l⁻¹), D-(+)-cellobiose (2 g l⁻¹) and a mixture of 20 amino acids (each at 0.1 g l⁻¹). This experiment was performed under a H_2 (100%, 200 kPa) gas phase. All these tests were performed in serum vials and positive cultures were transferred to confirm growth. Growth was determined by direct cell counts in a Thoma chamber (depth 0.02 mm) with a phase-contrast microscope.

Determination of H_2S . H_2S formation was detected by the addition of $500 \mu\text{l } 5 \text{ mM CuSO}_4/50 \text{ mM HCl}$ to 0.2 ml of the culture. A brown precipitate demonstrated the presence of H_2S .

Determination of ammonia. $\text{NH}_3/\text{NH}_4^+$ formation was analysed as described by Koroleff (1969), with the modifications reported by Aminot & Chaussepied (1983). This method is based on the formation of indophenol blue, which absorbs at 630 nm in the presence of NH_3 or NH_4^+ . For this experiment, cells were grown on KA22 medium prepared without sulphur and NO_3^- was therefore the sole electron acceptor. This test was carried out in duplicate and repeated twice.

Susceptibility to antibiotics. Sensitivity to antibiotics was estimated by using 25, 50, 75 and $100 \mu\text{g ml}^{-1}$ solutions of the following antibiotics: chloramphenicol, kanamycin, penicillin G, rifampicin, streptomycin and vancomycin. Antibiotic solutions were added to KA22 medium just before inoculation. When the antibiotic was diluted in ethanol (chloramphenicol) or DMSO (rifampicin), the same volume of solvent was added to control cultures.

DNA extraction and purification. Genomic DNA was extracted as described by Wery *et al.* (2001). The concentration and purity of the genomic DNA obtained were estimated by use of a GenQuant II spectrophotometer (Pharmacia) at 260, 280 and 320 nm. The quality of the extraction was checked on a 0.8% (w/v) agarose gel containing $0.5 \mu\text{g}$ ethidium bromide ml^{-1} .

DNA base composition. The DNA was purified by caesium chloride gradient centrifugation (Sambrook *et al.*, 1989) and its purity was checked spectrophotometrically. The G + C content of the genomic DNA was determined from the melting point according to Marmur & Doty (1962), under the conditions reported by Raguénès *et al.* (1997). A calibration curve was constructed by use of ultrapure DNA from *Escherichia coli* strain B (50 mol% G + C), *Clostridium perfringens* (26.5 mol% G + C) and *Micrococcus luteus* (72 mol% G + C) as standards (Sigma).

Amplification of the 16S rDNA. The 16S rDNA was selectively amplified from purified genomic DNA by PCR with oligonucleotide primers designed to anneal to conserved positions in the 3' and 5' regions of the 16S rRNA genes. The forward primer was SAdir (5'-AGAGTTTGATCATGGC-TCAGA-3'), corresponding to positions 8–28 in the *E. coli* 16S rRNA, and the reverse primer was S17rev (5'-GTTAC-CTTGTTACGACTT-3'), corresponding to positions 1493–1509. The initial denaturation step consisted of heating the reaction mixture to $94 \text{ }^\circ\text{C}$ for 3 min. This was followed by 30 cycles of denaturation at $94 \text{ }^\circ\text{C}$ for 1 min, annealing at $49 \text{ }^\circ\text{C}$ for 90 s and extension at $72 \text{ }^\circ\text{C}$ for 2 min. A final extension step was carried out at $72 \text{ }^\circ\text{C}$ for 6 min. The PCR products were analysed on 0.8% (w/v) agarose TAE gels (0.04 M Tris/acetate, 0.001 M EDTA) containing $0.8 \mu\text{g}$ ethidium bromide ml^{-1} and recorded with a Fluor-S multiimager (Bio-Rad).

16S rDNA sequence analysis. The PCR product was sequenced bidirectionally with the primers described by Raguénès *et al.* (1996) by Genome Express SA (Grenoble, France) with an automatic DNA analysis system (Applied Biosystems). Some 1414 positions of the 16S rDNA were determined. The 16S rDNA sequence of strain AM1116^T was compared to the sequences of representative proteobacteria and clones from hydrothermal vents. The CLUSTAL w method with weighted residues was used to align the sequences and to calculate similarity levels (Thompson *et al.*, 1994). Alignment was refined manually by use of the multiple sequence alignment editor SEAVIEW and phylogenetic reconstruction was achieved by use of PHYLO_WIN (Galtier *et al.*, 1996) with the following settings: Jukes-Cantor distance matrix and successively the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Lake, 1987) and maximum-likelihood (Felsenstein, 1981) methods. Some 1104 nucleotides were included in the phylogenetic analysis. Bootstrap values were determined according to Felsenstein (1985). *E. coli* K-12 (J01695), *Vibrio diabolus* CNCM I-1629^T (X99762) and the sequence of the uncultured symbiont of *Riftia pachyptila* (M99451) were used as outgroups.

RESULTS AND DISCUSSION

Enrichment and isolation

Enrichment cultures were performed on KA22 medium with entire young *Alvinella pompejana* tubes (cleaned of their inner contents) attached to small fragments of chimney rock. After 20 h incubation, growth was observed at $60 \text{ }^\circ\text{C}$ under a H_2/CO_2 (80:20; 200 kPa) gas phase. The enriched culture consisted of dense populations of short, rod-shaped, motile cells. It was subcultured successfully. One isolate was purified with five serial dilutions to extinction and is referred to as strain AM1116^T (= DSM 14510^T = CIP 107140^T). The purity of the isolate was confirmed by microscope observations and by cloning and sequencing of ten independent 16S rDNA clones.

Morphological and physiological characteristics

Scanning electron microscopy and phase-contrast microscopy indicated that cells of isolate AM1116^T were motile rods, about 1.0–1.5 μm long and 0.5 μm wide. The cells were Gram-negative and appeared to divide

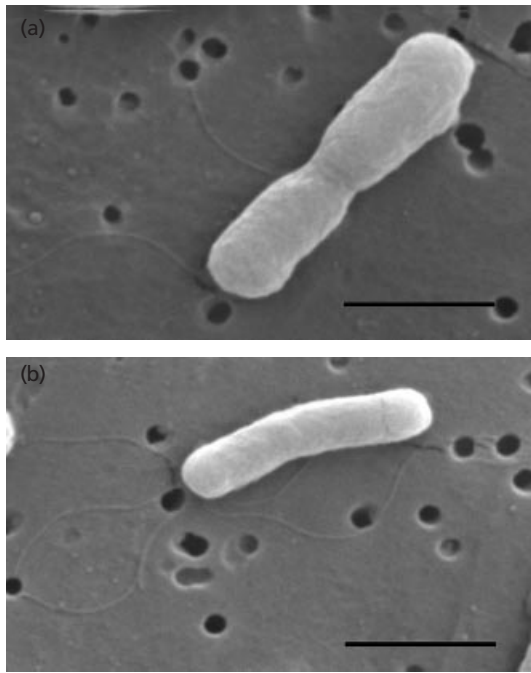


Fig. 1. Scanning electron micrographs of strain AM1116^T in the mid-exponential phase of growth, showing division by constriction (a) and polar flagella (b). Bars, 1 µm.

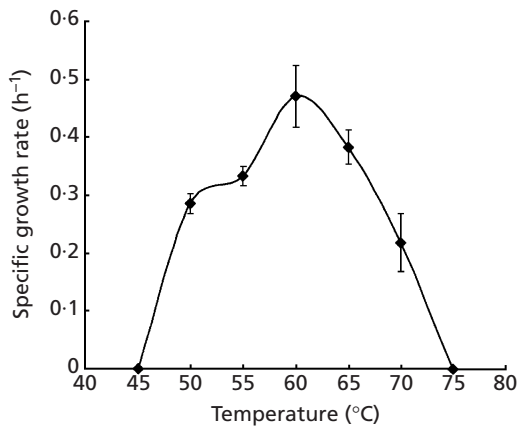


Fig. 2. Effect of temperature on the specific growth rate of strain AM1116^T. The cells were grown in KA22 medium (pH 6.0, 25 g NaCl l⁻¹). Growth rates were calculated by performing linear regression analysis along the logarithmic part of the growth curves.

by constriction (Fig. 1a). As observed during scanning electron microscope analyses (Fig. 1b) and confirmed by the SpotTest flagella stain kit (Difco) used for flagella detection, the flagella were polar. Strain AM1116^T grew at temperatures between 50 and 70 °C with optimum growth at around 60 °C; no growth was observed at 45 or 75 °C (Fig. 2). Strain AM1116^T required NaCl for growth; growth was observed at NaCl concentrations of between 10 and 40 g l⁻¹. The optimum NaCl concentration was between 20 and

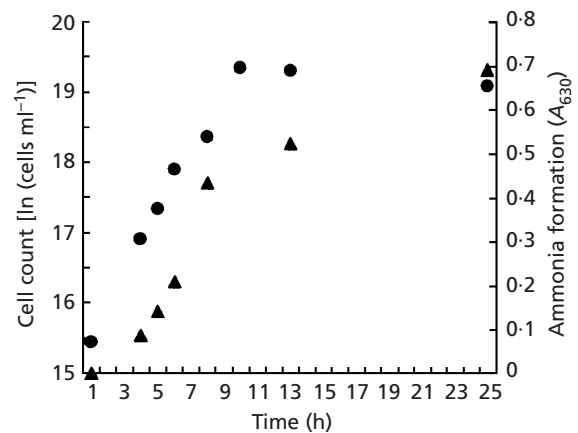


Fig. 3. Ammonia formation during the growth of isolate AM1116^T. The growth curve (●) and ammonia formation (▲) (determined by absorbance at 630 nm of indophenol blue, demonstrating the formation of NH₃/NH₄⁺) are shown.

25 g l⁻¹. Growth did not occur at 5 or 45 g NaCl l⁻¹. Growth occurred at between pH 5.0 and 7.5 and the optimum pH was around 5.5–6.0, which corresponded with the pH recorded in the *Alvinella pompejana* colony during sampling.

Metabolic properties

Strain AM1116^T was an anaerobic, obligately hydrogen-oxidizing bacterium, unable to grow under microaerophilic conditions. It was unable to grow organotrophically on the complex substrates or small organic molecules tested. Growth of the novel isolate was optimal in the presence of elemental sulphur or nitrate as the terminal electron acceptor (doubling times were equal). H₂S was produced as a result of sulphur reduction. When nitrate was the terminal electron acceptor, isolate AM1116^T produced high levels of ammonia or ammonium, which accumulated in the culture medium (Fig. 3). These data suggest that the newly isolated strain is an obligately chemolithotrophic bacterium that produces energy by reducing elemental sulphur or nitrate and by using molecular hydrogen as an electron donor. Of the other electron acceptors tested with H₂ as an electron donor and CO₂ as a carbon source, strain AM1116^T was found to reduce cystine slightly (production of H₂S), but not sulphate, thiosulphate or polysulphides. Very strong autotrophic growth occurred with CO₂ and weak heterotrophic growth occurred on yeast extract, tryptone, brain/heart infusion, peptone and gelatin. Limited growth observed on complex proteinaceous substrates probably resulted from decarboxylation of amino acids. The other carbon sources tested did not support growth. In optimum growth conditions (with CO₂ as a carbon source, H₂ as an electron donor, sulphur or nitrate as a terminal electron acceptor and at optimum temperature, pH and salinity), the final concentration was approximately 2.5 × 10⁸ cells ml⁻¹ in vials.

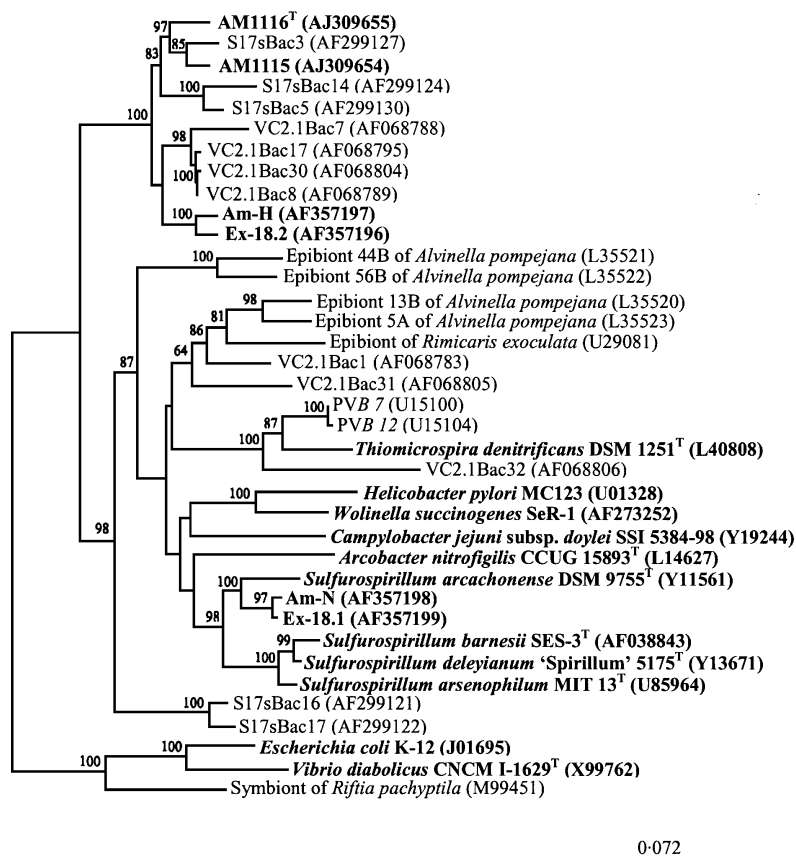


Fig. 4. Phylogenetic position of strain AM1116^T within the ϵ -Proteobacteria. The alignment was performed with 16S rDNA sequences of representative species of the ϵ -Proteobacteria (in bold) and uncultured ϵ -proteobacteria from Atlantic and Pacific hydrothermal vents. Three species from the γ -Proteobacteria were chosen as outgroups. Accession numbers and strains are noted in parentheses. The topology shown corresponds to an unrooted tree obtained by a neighbour-joining algorithm (Jukes & Cantor corrections) established using PHYLO_WIN. A total of 1104 nucleotides were included in the phylogenetic analysis. Bootstrap values are displayed on the branches. Bar, 7.2 nt substitutions per 100 nt. The positioning of the novel isolate was confirmed by maximum-parsimony and maximum-likelihood methods.

Susceptibility to antibiotics

Growth of isolate AM1116^T was inhibited by 25 μ g streptomycin, vancomycin, chloramphenicol, rifampicin and penicillin G ml⁻¹. The strain was resistant to 100 μ g kanamycin ml⁻¹.

DNA base composition

The G+C content of the genomic DNA of strain AM1116^T was 29 \pm 1 mol %.

Phylogenetic analysis and taxonomic position of the novel isolate

The almost-complete sequence (1414 bp) of the 16S rDNA of strain AM1116^T was determined. Phylogenetic analyses of the 16S rRNA gene located the strain within the ϵ -Proteobacteria, in the bacterial domain. The phylogenetic position of the organism was determined by comparing the 16S rDNA sequence of strain AM1116^T to those of 14 representative species of the ϵ -Proteobacteria and 19 16S rDNA sequences of uncultured members of the ϵ -Proteobacteria from Atlantic and Pacific hydrothermal vents. The three algorithms used (neighbour-joining, maximum-parsimony and maximum-likelihood) showed that the sequence of AM1116^T belonged to a lineage clearly separated from the lineage including the majority of the ϵ -Proteobacteria described (Fig. 4). This novel

lineage has been discovered recently by molecular methods (Polz & Cavanaugh, 1995; Moyer *et al.*, 1995; Reysenbach *et al.*, 2000; Longnecker & Reysenbach, 2001). It encompasses only bacteria from deep-sea Atlantic and Pacific vents.

The novel isolate was very closely related to several 16S rDNA sequences from deep-sea Atlantic vents (VC2.1Bac7, VC2.1Bac17, VC2.1Bac30, VC2.1Bac8 and several other sequences not marked on the phylogenetic tree; Reysenbach *et al.*, 2000) and south-eastern Pacific vents (S17sBac3, S17sBac14, S17sBac5 and several other sequences not marked on the phylogenetic tree; Longnecker & Reysenbach, 2001). They shared 92–95% 16S rDNA sequence similarity. Moreover, the strain was phylogenetically affiliated to two members of the ϵ -Proteobacteria found recently in enrichment cultures from the hydrothermal vent polychaete *Alvinella pompejana* from the East Pacific Rise 13° N (strain Am-H) and from chimney samples from the Guaymas basin (strain Ex-18.2) (Campbell *et al.*, 2001). The level of 16S rDNA sequence similarity between strain AM1116^T and these strains was 92–93%. Some of the metabolic properties of the novel isolate were similar to those of strains Am-H and Ex-18.2, which have been partially characterized and which are sulphur-reducing bacteria. Strain Am-H has been found to grow chemolithoautotrophically with H₂ as the electron donor and CO₂ as the carbon source, as is the case for strain AM1116^T. However, although

strain AM1116^T shared some characteristics with these two cultivable phylogenetic relatives, it also exhibited significant metabolic and physiological differences, notably its temperature range for growth. Strains Am-H and Ex-18.2 are moderate thermophiles (the optimum growth temperature of strain Am-H was 45 °C and the optimum growth temperature of strain Ex-18.2 has not been determined, but the strain was isolated at 45 °C). Moreover, these two isolates differed from strain AM1116^T in their ability to grow on formate as the energy and carbon source. In addition, strain Am-H has been found to grow chemoorganoheterotrophically on pyruvate as the electron donor and carbon source. If they belong to the same family as AM1116^T, strains Am-H and Ex-18.2 probably correspond to another genus.

Strain AM1116^T was closely related to strain AM1115 (95% 16S rDNA similarity), which was isolated in our laboratory (unpublished data) from the same sample as AM1116^T and under the same conditions except that the enrichment temperature was 45 °C. Similar to strain AM1116^T, this strain is a chemolithoautotrophic bacterium from the microenvironment of *Alvinella pompejana* that produces energy by using molecular hydrogen as an electron donor and by reducing elemental sulphur (unpublished data).

Although strain AM1116^T was isolated from a sample taken from the environment of *Alvinella pompejana*, it belongs to a phylotype that is very distantly related to the filamentous epibiotic ϵ -proteobacteria of this metazoan (Cary *et al.*, 1997) (mean similarity of 85–86%). Despite the fact that our isolate was found to be associated with the tubes of *Alvinella pompejana*, we hypothesize that it is not dependent on them.

On the basis of the overall 16S rRNA dissimilarity value of 17% between strain AM1116^T and the closest fully described relatives (members of the genus *Sulfurospirillum*) and the fact that strain AM1116^T and its relatives are distributed widely at deep-sea hydrothermal vents, we propose that strain AM1116^T should be assigned as the type strain of the type species of a novel genus within the ϵ -Proteobacteria. Because of its hydrothermal vent origin and its ability to grow lithotrophically on H₂, we have named the novel species *Caminibacter hydrogeniphilus* gen. nov., sp. nov. A novel family will have to be established in the future to encompass this genus and related genera yet to be described.

Ecological significance

As indicated by their dominance in the molecular surveys that have studied hydrothermal vent communities (Polz & Cavanaugh, 1995; Moyer *et al.*, 1995; Reysenbach *et al.*, 2000; Longnecker & Reysenbach, 2001), members of the ϵ -Proteobacteria certainly have a significant role in the ecology of hydrothermal vents. Their widespread distribution and autotrophic abilities suggest that strain AM1116^T and its relatives are probably a major source of primary productivity in

hydrothermal vents. Their ability to use H₂ as an electron donor and S⁰ as an electron acceptor may reflect the physiological remains of ancient metabolic pathways that first evolved in primitive organisms because of the ready availability of these inorganic compounds on the primitive Earth. Their ability to reduce elemental sulphur and nitrates in some cases enables them to contribute specifically to the sulphur and nitrogen cycles and more generally to biogeochemical cycling in deep-sea hydrothermal vents. Considering the wide geochemical gradients (nutrients, temperatures, oxygen etc.) present at deep-sea hydrothermal vents, the metabolic and physiological abilities of the ϵ -proteobacteria from these extreme environments are probably more diverse than has so far been described. Fully appreciating the diversity among the members of the ϵ -Proteobacteria and more widely among the entire microbial population in deep-sea environments is still a substantial challenge.

Description of *Caminibacter* gen. nov.

Caminibacter (Ca'mi.ni.bac.ter. L. gen. n. *camini* of a chimney; N.L. n. *bacter* masc. equivalent of Gr. neut. n. *bakterion* rod, staff; N.L. n. *Caminibacter* rod from a hydrothermal chimney, relating to the origin of the type species).

Cells are rod-shaped, motile and stain Gram-negative. They are thermophiles, adapted to the salinity of the ocean. Anaerobic, hydrogen-oxidizing, sulphur-reducing and denitrifying. Chemolithoautotrophic to mixotrophic. 16S rDNA sequence comparisons locate the genus within the ϵ -Proteobacteria in the bacterial domain. This genus is not closely related to any described genera. The type and only species is *Caminibacter hydrogeniphilus*.

Description of *Caminibacter hydrogeniphilus* sp. nov.

Caminibacter hydrogeniphilus (hy.dro.ge.ni.phil'us. N.L. *hydrogenum* hydrogen; Gr. n. *philos* friend; N.L. adj. *hydrogeniphilus* hydrogen-liking, referring to its ability to grow lithotrophically on H₂).

Cells are rod-shaped (1.0–1.5 × 0.5 µm), motile by polar flagella and stain Gram-negative. Cells divide by constriction. Growth occurs at between 50 and 70 °C (optimum 60 °C), pH 5.0 and 7.5 (optimum 5.5–6.0) and 10 and 40 g NaCl l⁻¹ (optimum 20–25 g NaCl l⁻¹). Doubling time under optimum growth conditions is around 1.5 h; maximum cell yield is 2.5 × 10⁸ cells ml⁻¹ in vials. Anaerobic. Obligately hydrogen-oxidizing. Chemolithoautotrophic to mixotrophic (heterotrophic growth on yeast extract, tryptone, peptone, brain/heart infusion and gelatin). Reduces S⁰ and nitrate and, to a lesser extent, cystine. The DNA G+C content of the type strain is 29 ± 1 mol%. The GenBank/EMBL accession number for the 16S rDNA sequence of the type strain is AJ309655.

The type strain, strain AM1116^T (= DSM 14510^T = CIP 107140^T), was isolated from young *Alvinella*

pompejana tubes attached to fragments of chimney rock that were collected from the hydrothermal site Elsa (HOT3) in the East Pacific Rise (103° 56' W, 12° 48' N).

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