Hydrogenimonas thermophila gen. nov., sp. nov., a novel thermophilic, hydrogen-oxidizing chemolithoautotroph within the ϵ -*Proteobacteria*, isolated from a black smoker in a Central Indian Ridge hydrothermal field

Ken Takai,¹ Kenneth H. Nealson^{1,2} and Koki Horikoshi¹

Correspondence

Ken Takai

kent@jamstec.go.jp

¹Subground Animalcule Retrieval (SUGAR) Project, Frontier Research System for
Extremophiles, Japan Marine Science and Technology Centre, 2-15 Natsushima-cho,
Yokosuka 237-0061, Japan

²Department of Earth Sciences, University of Southern California, 3651 Trousdale Parkway,
Los Angeles, CA 90089-0740, USA

A novel thermophilic bacterium, strain EP1-55-1%^T, was isolated from an *in-situ* colonization system deployed in a superheated, deep-sea, hydrothermal vent emission at the Kairei Field on the Central Indian Ridge in the Indian Ocean. The cells were highly motile rods, each possessing a single polar flagellum. Growth was observed between 35 and 65 °C (optimum temperature, 55 °C; 70 min doubling time) and between pH 4.9 and 7.2 (optimum, pH 5.9). The isolate was a microaerobic-to-anaerobic chemolithoautotroph capable of using molecular hydrogen as the sole energy source and carbon dioxide as the sole carbon source. Molecular oxygen, nitrate or elemental sulfur (S⁰) could serve as electron acceptors to support growth. The G + C content of the genomic DNA was 34.6 mol%. Phylogenetic analysis based on 16S rDNA sequences indicated that strain EP1-55-1%^T represents the first strain for which taxonomic properties have been characterized within the previously uncultivated phylogroup classified as belonging to the uncultivated ϵ -*Proteobacteria* group A; the name *Hydrogenimonas thermophila* gen. nov., sp. nov. is proposed, with strain EP1-55-1%^T (= JCM 11971^T = ATCC BAA-737^T) as the type strain.

During the last decade, molecular phylogenetic surveys of microbial ecosystems in global deep-sea hydrothermal systems have revealed a great diversity of micro-organisms inhabiting niches associated with steep physical and geochemical gradients (Takai & Fujiwara, 2002). In high-temperature habitats adjacent to hot, anoxic hydrothermal fluids, anaerobic, hyperthermophilic members of the *Archaea* and the *Bacteria* dominate the microbial communities, while, at lower temperatures, the predominant microbial components are psychrophilic and aerobic entities (Takai & Fujiwara, 2002). Analyses, using small-subunit rRNA gene (rDNA) sequences, of microbial communities in near-vent environments have revealed the predominance and diversity of previously uncultivated

members of the ϵ -*Proteobacteria* (Cary *et al.*, 1997; Corre *et al.*, 2001; Longnecker & Reysenbach, 2001; Moyer *et al.*, 1995; Reysenbach *et al.*, 2000). However, their physiology has remained unclear because of their resistance to cultivation.

Recently, isolation and characterization of previously uncultivated members of the ϵ -*Proteobacteria* have been reported (Campbell *et al.*, 2001; Miroshnichenko *et al.*, 2002; Alain *et al.*, 2002). *Nautilia lithotrophica* strain 525^T (Miroshnichenko *et al.*, 2002) and *Caminibacter hydrogeniphilus* strain AM1116^T (Alain *et al.*, 2002) were identified as novel strictly anaerobic, hydrogen-oxidizing, thermophilic chemolithomixotrophs from the nests of tube-dwelling polychaetes in deep-sea hydrothermal systems in the East-Pacific Rise. Other isolates (strains Am-H and EX-18.2) from similar habitats were reported as slightly thermophilic, anaerobic mixotrophs capable of using hydrogen as an energy source and elemental sulfur as a primary electron acceptor (Campbell *et al.*, 2001). These isolates represented the first cultivated members of the ϵ -*Proteobacteria* of previously uncultivated group D, one

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain EP1-55-1%^T is AB105049.

Details of the effects of temperature, pH and NaCl concentration on the growth of strain EP1-55-1%^T are available as supplementary data in IJSEM Online.

of the phylogenetic groups within the *ε-Proteobacteria* according to the classification by Corre *et al.* (2001). Our laboratory has recently cultivated and partially characterized many isolates of the *ε-Proteobacteria*, representing nearly all of the previously uncultivated subgroups inhabiting deep-sea hydrothermal environments (Takai *et al.*, 2003a). In the investigation, thiosulfate and elemental sulfur as well as molecular hydrogen were provided together as energy sources together with molecular oxygen and nitrate as electron acceptors, which are more abundant than elemental sulfur in the ambient habitats of deep-sea hydrothermal systems (Takai *et al.*, 2003a). On the basis of its partial 16S rDNA sequence, one of the thermophilic strains, designated EP1-55-1%^T, was phylogenetically affiliated with the uncultivated *ε-Proteobacteria* group A, a thermophilic lineage completely different from the previously described *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) and *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002). Here, we report a taxonomic study and the partial characterization of strain EP1-55-1%^T and propose the new genus *Hydrogenimonas*.

Sample collection, enrichment and purification

Sample collection and subsampling procedures were as described previously (Takai *et al.*, 2003a). An *in-situ* colonization system, a new artificial microbial habitat consisting of a stainless steel pipe with many small holes (5 mm in diameter) and a substratum of very porous, natural and synthetic pumice (Takai *et al.*, 2003a), was deployed for 3 days in a black smoker vent orifice with a vent emission temperature of >250 °C at the Kairei Field on the Central Indian Ridge (25°19'23'S, 70°02'42'E), at a depth of 2421 m. After deployment, it was recovered by the manned submersible *Shinkai 6500*. The Kairei hydrothermal field on the Central Indian Ridge of the Indian Ocean was discovered for the first time by Japanese scientists in 2000 (Hashimoto *et al.*, 2001), and the physical and chemical characterization of the vent emissions was described elsewhere (Gamo *et al.*, 2001). The substratum of the *in-situ* colonization system was suspended in 20 ml sterilized MJ synthetic sea water (Sako *et al.*, 1996; Takai *et al.*, 1999) containing 0.05 % (w/v) sodium sulfide in a 100 ml glass bottle (Schott Glaswerke) tightly sealed with a butyl-rubber cap under a gas phase of 100 % N₂ (100 kPa). The suspended slurry was used to inoculate a series of media, including MMJHS medium (Takai *et al.*, 2003a), under a gas phase of H₂/CO₂/O₂ (80:19:1, by vol.; 300 kPa), and the cultures were incubated at 55 °C in a dry oven.

Growth of thermophiles was observed in MMJHS medium after 2 days incubation at 55 °C. Enrichment cultures at 55 °C contained rod-shaped cells. A pure culture was obtained by using the dilution-to-extinction technique at 55 °C with the same medium as that used for the enrichment (Takai & Horikoshi, 2000). The culture in the tube showing growth at the highest dilution was designated strain EP1-55-1%^T (=JCM 11971^T=ATCC BAA-737^T). Purity was confirmed routinely by microscopic examination

and by repeated partial sequencing of the 16S rRNA gene using several PCR primers.

Morphology

Cells were observed under a phase-contrast Olympus BX51 microscope with the SPOT RT Slider CCD camera system (Diagnostic Instruments). Transmission electron microscopy of negatively stained cells and thin sections of cells was carried out as described by Zillig *et al.* (1990) and Takai *et al.* (1999). Cells grown in MMJH medium (described below) under microaerobic conditions (1 % partial pressure of O₂) at 55 °C in the mid-exponential phase of growth were used for microscopic observation. Cells of strain EP1-55-1%^T were Gram-negative rods, about 0.7–1.0 µm in diameter, 1.5–3.5 µm long (Fig. 1a, c) and highly motile by means of a polar flagellum (Fig. 1a). When strain EP1-55-1% was grown in MMJH medium under anaerobic conditions, cells became uniformly non-motile and spherical in the stationary phase. A small percentage of cells grown under microaerobic conditions also exhibited this spherical shape (Fig. 1b) in the stationary phase. Although similar morphological features were reported for *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) and *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002) (Table 1), the formation of a spherical cell shape in strain EP1-55-1%^T was a distinctive morphological feature with respect to these previously described thermophilic *ε*-proteobacterial genera.

Growth characteristics

Strain EP1-55-1% was routinely cultivated in MMJH medium. MMJH medium was MMJ medium (Takai *et al.*, 2002) supplemented with 5 mM calcium chloride (CaCl₂) and 10 mM sodium nitrate (NaNO₃) without sodium sulfide (Na₂S.9H₂O) or cysteine hydrochloride. To prepare the medium, everything except the vitamin solution (Balch *et al.*, 1979) and NaHCO₃ was dissolved and the pH of the medium was adjusted to around 6.0 with HCl before autoclaving. After autoclaving under an air atmosphere, a concentrated solution of vitamins and NaHCO₃ was added to the medium under gas purging with H₂/CO₂ (80:20, v/v), and the pH was readjusted to 6.0 with HCl at room temperature, if necessary. A concentrated sodium bicarbonate solution was separately sterilized by autoclaving, and the vitamin solution was filter-sterilized. The medium was dispensed to 20 % of the bottle (Schott Glaswerke) or tube (Iwaki Glass) volume and tightly sealed with a butyl-rubber stopper under a gas phase of H₂/CO₂/O₂ (80:19:1, by vol.) at 300 kPa unless otherwise noted.

Growth of strain EP1-55-1%^T was measured by direct cell counting after staining with 4',6-diamidino-2-phenylindole (Porter & Feig, 1980) using a phase-contrast Olympus BX51 microscope. Cultures were prepared in duplicate in 100 ml glass bottles (Schott Glaswerke), each containing 20 ml medium, with shaking (100 r.p.m.) in a temperature-controlled dry oven. With MMJH medium, strain EP1-55-1%^T grew over the temperature range of about 35–65 °C,

Fig. 1. Electron micrographs of a negatively stained cell (a, b) and a thin-section of a cell (c) of strain EP1-55-1%^T. (a, c) Rod cell in the mid-exponential phase of growth. (b) Spherical cells observed in the stationary phase of culture under anaerobic conditions. CM, Cellular membrane; OM, outer membrane. Bars, 0.5 μm .

showing optimal growth at 55 °C, and the generation time at 55 °C, pH 6.0, was about 70 min (see Supplementary Figure in IJSEM Online). The effect of pH on growth was tested at 55 °C, using MMJH medium adjusted to various values with 10 mM acetate/acetic acid buffer (pH 4–5), MES (pH 5–6), PIPES (pH 6–7), HEPES (pH 7–7.5) and Tris (pH 8–9.5) at room temperature (Supplementary Figure). Growth occurred at pH 4.9–7.2, with optimum growth at about pH 5.9 (Supplementary Figure). No growth was observed below pH 4.5 or above pH 7.7. The pH was found to be stable during the cultivation period. Strain EP1-55-1%^T, when tested in MMJH medium with

variable amounts of added NaCl, grew at NaCl concentrations in the range 16–56 g l⁻¹, with optimum growth at 32 g l⁻¹ NaCl at 55 °C and pH 6.0 (Supplementary Figure). In general, the temperature and salt requirements of EP1-55-1%^T were similar to those of *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) and *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002) (Table 1).

The effect of the oxygen concentration in the gas phase on growth of strain EP1-55-1%^T was tested with MMJH medium under the following gas mixtures (at 300 kPa, all by volume): H₂/CO₂, 80:20; H₂/CO₂/O₂, 80:19.9:0.1; H₂/CO₂/O₂, 80:19.5:0.5; H₂/CO₂/O₂, 80:19:1; H₂/CO₂/O₂, 80:18:2; or H₂/CO₂/O₂, 80:17:3. The maximum cell yield of strain EP1-55-1%^T was approximately 3 × 10⁹ cells ml⁻¹ under a gas phase in the absence or presence of up to 1% O₂; a slightly lower yield (8 × 10⁸ cells ml⁻¹) was seen with 2% O₂. No growth was observed with 3% O₂ in the gas phase. These results indicated that strain EP1-55-1%^T grows under anaerobic-to-microaerobic (up to 2% O₂) conditions.

Heterotrophic growth was tested in MMJH medium without NaHCO₃ under a gas phase of H₂/O₂ (99:1, v/v; 300 kPa), containing the following potential carbon sources: yeast extract, peptone, tryptone, Casamino acids (all 0.2%, w/v), formate, acetate, glycerol (all 5 mM), 0.025% (v/v) methanol, 0.05% (v/v) ethanol, 0.1% (v/v) 2-propanol, citrate, tartrate, fumarate, malate, succinate, propionate, maleate, lactate, oxalate, thioglycolate, pyruvate (all 5 mM), 5 mM of each of 20 amino acids, glucose, galactose, sucrose, fructose, lactose, maltose, arabinose, trehalose and starch (all 0.1%, w/v). Strain EP1-55-1%^T was not able to grow with any of the heterotrophic substrates, using H₂ as an energy source and nitrate or molecular oxygen as an electron acceptor.

Utilization of these organic compounds as alternative energy sources instead of H₂ was also examined in MMJH medium under a gas phase of N₂/CO₂/O₂ (80:19:1, by vol.; 300 kPa). None of the organic compounds sustained growth of strain EP1-55-1%^T. In an attempt to determine potential electron donors other than H₂ for autotrophic growth, sulfide (5 or 20 mM), thiosulfate (20 mM), sulfite (5 or 20 mM), elemental sulfur (3%, w/v) and ferrous iron (20 mM) were tested instead of H₂ as sole electron donors in MMJH medium with a gas phase of N₂/CO₂/O₂ (80:19:1, by vol.; 300 kPa). For testing utilization of electron acceptors, molecular oxygen (0.1 or 1% O₂ in the gas phase) was examined with MMJH medium in the absence of nitrate under a H₂/CO₂ gas mixture and nitrate (10 mM), nitrite (1 or 5 mM), sulfite (2 or 10 mM), thiosulfate (10 mM), tetrathionate (10 mM), ferric citrate (20 mM), ferrihydrite (20 mM), selenate (5 mM), arsenate (5 mM) or fumarate (10 mM) were tested with MMJH medium under H₂/CO₂ (80:20, v/v; 300 kPa). None of the electron donors other than H₂ supported growth of strain EP1-55-1%^T, while molecular oxygen (1% O₂) or nitrate (10 mM) was able to serve as the primary electron acceptor,

Table 1. Comparison of properties among *Hydrogenimonas thermophila* gen. nov., sp. nov., *N. lithotrophica* and *C. hydrogeniphilus*

Strains: 1, *H. thermophila* EP1-55-1%^T; 2, *N. lithotrophica* 525^T (data from Miroshnichenko *et al.*, 2002); 3, *C. hydrogeniphilus* AM1116^T (data from Alain *et al.*, 2002). ND, Not determined.

Characteristic	1	2	3
Cell shape	Short rod or spherical	Short rod	Short rod
Temperature for growth: (°C)			
Range	35–65	37–68	50–70
Optimum	55	53	60
Doubling time under optimal conditions (min)	70	140	90
pH for growth:			
Range	4.9–7.2	6.4–7.4	5.5–7.5
Optimum	5.9	6.8–7.0	5.5–6.0
NaCl concentration for growth: (% w/v)			
Range	1.6–5.6	0.8–5.0	1.0–4.0
Optimum	3.2	3	2.0–2.5
Microaerobic growth	+ (up to 2 % O ₂)	–	–
Carbon sources other than CO ₂ :			
Formate	–	+	–
Complex organic substrates	–	–	+
Electron donors other than H ₂ :			
Formate	–	+	–
Electron acceptors other than S ⁰ :			
O ₂	+	–	–
Nitrate	+	–	+
Sulfite	–	+	–
Cystine	–	–	+
Nitrogen sources:			
Ammonium	+	+	ND
Nitrate	+	+	ND
G+C content of genomic DNA (mol%)	34.6	34.7	29

providing a maximum cell yield of 3×10^9 cells ml⁻¹. Elemental sulfur (3 %, w/v) gave a slightly lower cell yield (1×10^9 cells ml⁻¹). Potential nutrients required for growth, such as selenite, tungstate and vitamins, were examined with MMJH medium with and without the specified nutrients. The nitrogen source (NH₄Cl, NaNO₂, N₂ or NaNO₃) for growth was also examined with MMJH medium lacking all nitrogen sources. Strain EP1-55-1%^T utilized nitrate and ammonium as nitrogen sources and not nitrite or molecular nitrogen. Selenium, tungsten and vitamins were not required for growth. However, when the concentration of calcium chloride in MMJH medium was reduced from 0.08 to 0.01 % (w/v), the maximum cell yield of strain EP1-55-1%^T was reduced to 8.0×10^8 cells ml⁻¹. A relatively high concentration of calcium was stimulatory for growth of strain EP1-55-1%^T. These results indicated that strain EP1-55-1%^T is a strict chemolithoautotroph utilizing hydrogen as the sole energy source, molecular oxygen (up to 2 %, v/v), nitrate and elemental sulfur as electron acceptors and carbon dioxide as the sole carbon source for growth. The microaerobic growth and utilization of molecular oxygen as an electron acceptor by strain EP1-55-1%^T represent a marked difference from the strictly anaerobic

energy metabolisms of *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) and *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002) (Table 1).

The time-course of reduction of nitrate and elemental sulfur and the concomitant bacterial growth of strain EP1-55-1%^T were examined with MMJH medium under anaerobic conditions in the absence of ammonium and nitrate (Fig. 2). The concentrations of nitrate and nitrite were analysed by ion chromatography using a Shim-pack IC column (Shimadzu); the production of N₂, N₂O and H₂S in the gas phase during growth was monitored using a Micro GC CP2002 gas chromatograph (GL Sciences). Nessler's reagent was employed to measure the ammonium-ion concentration in the medium (Allen *et al.*, 1974). Consumption of nitrate and production of ammonium occurred during growth of strain EP1-55-1%^T when nitrate was given as the sole electron acceptor (Fig. 2). Production of nitrite, N₂ and N₂O was not observed during growth. Hydrogen sulfide was accumulated in the gas phase depending on growth with elemental sulfur as sole electron acceptor (data not shown). Control (uninoculated) medium did not exhibit either nitrate or elemental sulfur reduction.

Fig. 2. Time-course of the reduction of nitrate (□), the production of ammonium (▲) and concomitant bacterial growth (●) of strain EP1-55-1%^T.

Thus, strain EP1-55-1%^T was found to be a respiratory hydrogen-oxidizing, oxygen-, nitrate- or sulfur-reducing chemolithoautotroph.

Strain EP1-55-1%^T was found to be sensitive to a variety of antibiotics, including chloramphenicol, streptomycin, kanamycin, ampicillin and rifampicin (all at 50 µg ml⁻¹; data not shown). Antibiotic susceptibility was similar for strain EP1-55-1%^T and *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002), but *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002) was resistant to kanamycin (100 µg ml⁻¹).

Fatty acid analysis

The cellular fatty acid composition was analysed with cells grown in MMJH medium at 55 °C in the late-exponential growth phase. Lyophilized cells (100 mg) were placed in a Teflon-lined, screw-capped tube containing 3 ml anhydrous methanolic HCl and heated at 100 °C for 3 h. Extraction and analysis of fatty acid methyl esters were as described previously (Takai *et al.*, 2003b). The major cellular fatty acids of strain EP1-55-1%^T were C_{11:0} (2.2%), C_{12:0} (3.0%), C_{14:0} (4.7%), 3-OH-C_{14:0} (2.8%), C_{16:0} (37.4%), C_{16:1} (28.8%), C_{18:0} (1.0%) and C_{18:1} (20.0%). This composition was similar to that of a recently described mesophilic isolate, *Sulfurimonas autotrophica* OK-10^T, from a deep-sea hydrothermal system in the Okinawa Trough (Inagaki *et al.*, 2003).

Nucleic acid analyses

Genomic DNA of strain EP1-55-1%^T was prepared as described by Marmur & Doty (1962). The G + C content of the DNA was determined by direct analysis of deoxyribonucleotides by using HPLC (Tamaoka & Komagata, 1984). The G + C content of the genomic DNA of strain EP1-55-1%^T was 34.6 mol%, which is similar to that of *N. lithotrophica* 525^T (34.7 mol%; Miroshnichenko *et al.*,

2002) but higher than that of *C. hydrogeniphilus* AM1116^T (29 mol%; Alain *et al.*, 2002) (Table 1).

The 16S rRNA gene (rDNA) was amplified by PCR using primers Bac 27F and 1492R (DeLong, 1992; Lane, 1985), as described previously (Takai *et al.*, 2001). The nearly complete 16S rDNA sequence (1422 bp) of strain EP1-55-1%^T was obtained by direct sequencing of both strands, using the dideoxynucleotide chain-termination method with a DNA sequencer (model 3100; Perkin Elmer/Applied Biosystems). The rDNA sequence was analysed using the gapped-BLAST search algorithm (Altschul *et al.*, 1997; Benson *et al.*, 1998) and found to be most closely related (99.7%) to the sequences of strain EP365-55-1% (isolated from the same deep-sea hydrothermal field; Takai *et al.*, 2003a) and strain BKB55-1 [94.4%; isolated from the Iheya North site in the Okinawa Trough (Takai *et al.*, 2003a)] and to have similarity to sequences of the slightly thermophilic *Sulfurospirillum* strain EX-18.1 (91.2%) and strain Am-N (90.8%) (isolated from East-Pacific Rise deep-sea hydrothermal vent sites; Campbell *et al.*, 2001). Strain EP1-55-1%^T showed only a distant relationship (less than 90% similarity) to any strain of presently known members of the *ε-Proteobacteria*. The nearly complete sequence was realigned manually to 16S rDNA data from the Ribosomal Database Project II (Maidak *et al.*, 2000) on the basis of the alignments determined using the SEQUENCE ALIGNER program of Ribosomal Database Project II. Phylogenetic analyses were restricted to nucleotide positions that could be aligned unambiguously. Evolutionary distance-matrix analysis (using the Kimura two-parameter method, the least-squares distance method and a transition/transversion rate of 2.0) and neighbour-joining analysis were performed using the PHYLIP package, version 3.5 (Felsenstein, 1993) (Fig. 3). Bootstrap analysis was performed to provide confidence estimates for phylogenetic tree topologies. The phylogenetic tree indicated that strain EP1-55-1%^T represented the first strain for which taxonomic properties have been characterized within the previously uncultivated phylogroup classified within the uncultivated group A *ε-proteobacteria* (Corre *et al.*, 2001; Takai *et al.*, 2003a) (Fig. 3).

Comparison with related genera

Phylogenetic analysis indicates that strain EP1-55-1%^T is distantly related to any previously described members of the *ε-Proteobacteria*. Other than strain EP1-55-1%^T, several deep-sea hydrothermal vent *ε-proteobacteria* from the Central Indian Ridge Kairei hydrothermal field and the Okinawa Trough Iheya North site are affiliated with this phylogroup, together with a variety of environmental rDNA clones obtained from the Mid-Atlantic-Ridge deep-sea hydrothermal system (Takai *et al.*, 2003a). All thermophilic strains capable of growth at 55 °C isolated to date from deep-sea hydrothermal environments are classified within either group A or group D (Takai *et al.*, 2003a). Hence, these phylogroups might be characterized as a thermophilic lineage within the *ε-Proteobacteria*, representing at

Fig. 3. Phylogenetic tree of representative *ε*-proteobacterial strains and environmental rDNA clones potentially related to strain EP1-55-1%^T, inferred from 16S rDNA sequences by using the neighbour-joining method on 1011 homologous sequence positions for each organism. Numbers at nodes represent bootstrap values (100 replicates). The scale bar indicates 2 substitutions per 100 nucleotides.

least a new family level of taxa indigenous to the global deep-sea hydrothermal systems.

Only two thermophilic members of the *ε*-Proteobacteria, *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) and *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002), have been described previously, and, on the basis of both phylogenetic characterization and physiological properties, strain EP1-55-1%^T can be easily distinguished from them (Table 1). The genera *Nautilia* and *Caminibacter* are composed of strictly anaerobic mixotrophs capable of utilizing organic compounds as carbon sources. *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) uses formate as an energy source. Strain EP1-55-1%^T is able to grow under microaerobic conditions (up to 2% O₂) and is a strictly hydrogen-oxidizing chemolithoautotroph. Although all three thermophiles can use molecular hydrogen as the primary energy source, their utilization profiles for electron acceptors differ. The electron acceptors for strain EP1-55-1%^T are oxygen, nitrate and elemental sulfur. *C. hydrogeniphilus* AM1116^T (Alain *et al.*, 2002) can utilize elemental

sulfur, nitrate and, to lesser extent, cystine, while *N. lithotrophica* 525^T (Miroshnichenko *et al.*, 2002) primarily uses elemental sulfur or, alternatively, sulfite (Table 1). These are probably strong metabolic characteristics that differentiate strain EP1-55-1%^T from the genera within group D of the *ε*-Proteobacteria. On the basis of these physiological and molecular properties of strain EP1-55-1%^T, we propose a new genus, *Hydrogenimonas* gen. nov., with the type species *Hydrogenimonas thermophila* sp. nov.

Description of *Hydrogenimonas* gen. nov.

Hydrogenimonas (Hy.dro.ge.ni.mo'nas. N.L. neut. n. *hydrogenium* hydrogen; Gr. n. *monas* a unit, monad; N.L. fem. n. *Hydrogenimonas* hydrogen monad).

Short rods, highly motile via a polar flagellum. Gram-negative. Anaerobic to microaerobic. Thermophilic. Strictly chemolithoautotrophic. Able to utilize molecular hydrogen as an electron acceptor and molecular oxygen, nitrate or elemental sulfur as electron acceptors. NaCl absolutely

required for growth. Relatively high concentrations of calcium stimulate growth. The G+C content of the genomic DNA is about 35 mol%. Major cellular fatty acids are C_{16:0}, C_{16:1} and C_{18:1}. On the basis of 16S rRNA gene analysis, the genus *Hydrogenimonas* is distantly related to any previously described genera within the ϵ -*Proteobacteria*, representing a potential new family level of taxon. Members of the genus occur in global, deep-sea, hydrothermal systems. The type species is *Hydrogenimonas thermophila*.

Description of *Hydrogenimonas thermophila* sp. nov.

Hydrogenimonas thermophila (ther.mo'phil.a. Gr. adj. *thermus* hot; Gr. adj. *philos* loving; N.L. fem. adj. *thermophila* heat-loving).

Exhibits the following properties in addition to those given in the genus description. Cells have a mean length of 1.5–3.5 μ m and a width of approx. 0.7–1.0 μ m. Cells occur singly. Tolerates up to 2 % O₂ in the gas phase. The temperature range for growth is 35–65 °C (optimum at 55 °C). The pH range for growth is 4.9–7.2 (optimum at pH 5.9). NaCl in the concentration range 16–56 g l⁻¹ is an absolute growth requirement; optimum growth occurs at 32 g l⁻¹. Nitrate and elemental sulfur are respectively reduced to ammonium and hydrogen sulfide during growth. Nitrate or ammonium is required as a nitrogen source. Vitamins, selenium and tungsten are not required for growth. The major cellular fatty acids are C_{11:0} (2.2 %), C_{12:0} (3.0 %), C_{14:0} (4.7 %), 3-OH-C_{14:0} (2.8 %), C_{16:0} (37.4 %), C_{16:1} (28.8 %), C_{18:0} (1.0 %) and C_{18:1} (20.0 %). The G+C content of the DNA is 34.6 mol% (by HPLC).

The type strain, EP1-55-1%^T (= JCM 11971^T = ATCC BAA-737^T), was isolated from an *in-situ* colonization system deployed in the black smoker vent emission (> 250 °C) at the Kaiei Field on the Central Indian Ridge, Indian Ocean.

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