Sulfurihydrogenibium kristjanssonii sp. nov., a hydrogen- and sulfur-oxidizing thermophile isolated from a terrestrial Icelandic hot spring

Gilberto E. Flores,¹ Yitai Liu,¹ Isabel Ferrera,¹ Terry J. Beveridge² and Anna-Louise Reysenbach¹

Correspondence

Anna-Louise Reysenbach
reysenbacha@pdx.edu

¹Department of Biology, Portland State University, PO Box 751, Portland, OR 97207-0751, USA

²Department of Molecular and Cellular Biology, College of Biological Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada

Three thermophilic, aerobic, hydrogen- and sulfur-oxidizing bacteria were isolated from an Icelandic hot spring near the town of Hveragerdi and share >99% 16S rRNA gene sequence similarity. One of these isolates, designated strain I6628^T, was selected for further characterization. Strain I6628^T is a motile rod, 1.5–2.5 µm long and about 0.5 µm wide. Growth occurred between 40 and 73 °C (optimally at 68 °C), at pH 5.3–7.8 (optimally at pH 6.6) and at NaCl concentrations between 0 and 0.5% (w/v). Strain I6628^T grew with H₂, S⁰ or S₂O₃²⁻ as an electron donor with O₂ (up to 25%, v/v; optimally at 4–9%) as the sole electron acceptor. CO₂ and succinate were utilized as carbon sources but no organic compounds, including succinate, could be used as an energy source. The G + C content of the genomic DNA was determined to be 28.1 mol%. Phylogenetic analysis of the 16S rRNA gene sequence indicated that strain I6628^T is a member of the genus *Sulfurihydrogenibium*, the closest cultivated relative being the recently described strain *Sulfurihydrogenibium rodmanii* UZ3-5^T (98.2% sequence similarity). On the basis of the physiology and phylogeny of this organism, strain I6628^T represents a novel species of the genus *Sulfurihydrogenibium*, for which the name *Sulfurihydrogenibium kristjanssonii* sp. nov. is proposed. The type strain is I6628^T (=DSM 19534^T =OCM 901^T =ATCC BAA-1535^T).

The order *Aquificales*, which comprises thermophiles belonging to the families *Aquificaceae*, *Desulfurobacteriaceae* and *Hydrogenothermaceae*, is considered to be one of the deepest branching lineages of the domain *Bacteria* (Burggraf *et al.*, 1992; Pitulle *et al.*, 1994; Di Giulio, 2003a, b, c; Barion *et al.*, 2007). Members of two of these families, the *Aquificaceae* and the *Hydrogenothermaceae*, are widespread in terrestrial hydrothermal systems and include those belonging to the genera *Hydrogenobaculum*, *Hydrogenobacter*, *Sulfurihydrogenibium* and *Thermocrinis* (Reysenbach *et al.*, 2000; Skirnisdottir *et al.*, 2000; Takacs *et al.*, 2001; Spear *et al.*, 2005; Purcell *et al.*, 2007; Hetzer *et al.*, 2007; Ferrera *et al.*, 2007). Most representatives of these genera appear as filamentous biomass in hot-spring streams and are often associated with sulfur or iron deposits (Reysenbach *et al.*, 2005). Recently, several members of the genus *Sulfurihydrogenibium* have

been isolated from hydrothermal systems in geographically diverse locations, including Japan (*Sulfurihydrogenibium subterraneum* HGMK-1^T; Takai *et al.*, 2003), the Azores (*Sulfurihydrogenibium azorense* Az-Fu1^T; Aguiar *et al.*, 2004), Yellowstone National Park (USA) (*Sulfurihydrogenibium yellowstonense* SS-5^T; Nakagawa *et al.*, 2005), New Zealand (strain CP.B2; Hetzer *et al.*, 2007) and Kamchatka, Russia (*Sulfurihydrogenibium rodmanii* UZ3-5^T; O'Neill *et al.*, 2008). These studies have identified physiological properties common among members of this genus; several differences have also been revealed. For example, all described isolates are able to oxidize S⁰ and S₂O₃²⁻ with O₂ acting as an electron acceptor, but only *S. azorense* Az-Fu1^T, *S. subterraneum* HGMK-1^T and strain CP.B2 can also oxidize H₂ (Takai *et al.*, 2003; Aguiar *et al.*, 2004; Nakagawa *et al.*, 2005; Hetzer *et al.*, 2007; O'Neill *et al.*, 2008). Clone libraries of 16S rRNA gene sequences from Icelandic hot-spring filaments identified relatives of *Sulfurihydrogenibium* (Skirnisdottir *et al.*, 2000), and here we report the isolation of a novel member of the genus *Sulfurihydrogenibium* obtained from Iceland. The isolation of this novel species serves to expand the known geographical distribution and metabolic diversity of the genus *Sulfurihydrogenibium*.

The GenBank/EMBL/DDBJ accession number for the partial 16S rRNA gene sequence of strain I6628^T is AM778960.

Graphs showing the effects of temperature and pH on growth of strain I6628^T are available as supplementary figures with the online version of this paper.

Thick, grey filaments were collected along the outflow channel of an Icelandic hot spring near the town of Hveragerdi (64° 0.1901' N 21° 11.606' W) and transferred into sterile 150 ml serum bottles. The temperature and pH at the sampling site were 68 °C and approximately 6.0. Subsamples were later used to enrich for microaerophilic sulfur-oxidizers in 5 ml modified MS medium, described below (Boone *et al.*, 1989). Enrichment cultures were incubated at 70 °C and monitored for changes in turbidity. Within 24 h, cultures appeared turbid and were examined for growth by using phase-contrast microscopy. Several of the cultures, i.e. those comprising strains I6628^T, I66735 and I6517, exhibited growth in the form of motile rods. Cultures were subsequently purified by several series of dilution-to-extinction transfers and their purity was verified by 16S rRNA gene sequencing. The three isolates were >99 % identical on the basis of partial 16S rRNA gene sequences, and strain I6628^T was selected for further characterization.

The medium used for the isolation and characterization of strain I6628^T was modified MS medium (Boone *et al.*, 1989) and contained the following (l⁻¹): 5 g elemental sulfur, 0.8 g NaOH, 0.48 g KCl, 1 g MgCl₂·6H₂O, 7 g MgSO₄·7H₂O, 2 g Na₂S₂O₃·5H₂O, 0.48 g CaCl₂·2H₂O, 0.2 g NH₄Cl, 0.4 g K₂HPO₄·3H₂O and 10 ml trace element stock solution (Ferguson & Mah, 1983). The medium was prepared with distilled water under constant bubbling with CO₂. The pH was adjusted to 6.0 with sulfuric acid prior to autoclaving at 105 °C for 60 min to prevent melting of the elemental sulfur. After autoclaving, oxygen was added to a final concentration of 4 % (v/v).

Routine observations of the three isolates were made using an Olympus BX60 phase-contrast microscope. Further detailed examination of strain I6628^T was done using electron microscopy as described previously (Nakagawa *et al.*, 2005). In brief, for thin sectioning, cells were fixed in 2 % (v/v) glutaraldehyde followed by 2 % (w/v) osmium tetroxide and staining en bloc with 2 % (w/v) uranyl acetate, as outlined by Beveridge *et al.* (2007). Cells were dehydrated through an ethanol series and embedded in LR White resin. Once sectioned, cell samples were mounted on carbon- and Formvar-coated 200-mesh grids and stained with uranyl acetate and lead citrate. For negative stains, the grids were coated with a thin suspension of cells, blotted dry and stained with 2 % uranyl acetate. All transmission electron microscopy was done with a Philips CM10 microscope operating at 80 kV under standard operating conditions.

As shown by the thin sections, cells of strain I6628^T were Gram-negative, motile, straight to slightly curved rods with a mean length of 1.5–2.5 µm and a width of approximately 0.5 µm (Fig. 1a). Cells sometimes possessed humps along their length, indicating some pleomorphism (Fig. 1b). Cells occurred singly or in filaments, each consisting of a few cells (not shown). Flagella were apparent on negatively stained cells, especially at the poles (Fig. 1a). Thin sections

Fig. 1. Electron micrographs of a negatively stained cell (a) and a thin section (b) of strain I6628^T. Internal membranes or fibres are indicated by arrows in (b). Bars, 1 µm (a) and 500 nm (b).

also revealed the presence of internal stacked membranes or fibres, as has been reported for other members of the *Aquificales* (Götz *et al.*, 2002; Aguiar *et al.*, 2004) (Fig. 1b). However, the function of these structures remains unclear.

Growth of the isolate was monitored from direct cell counts using a Petroff–Hauser counting chamber viewed with phase-contrast microscopy. All characterization experiments were conducted in sealed 25 ml Balch tubes containing 5 ml modified MS medium inoculated from fresh overnight cultures at 5 % (v/v). Temperature, pH, electron donor/acceptor and carbon-substrate experiments were conducted in triplicate, while O₂ and NaCl experiments were conducted in duplicate. Unless stated otherwise, all cultivation experiments were conducted in a 68 °C water bath, at pH 6.0 and 4 % O₂ (v/v).

Growth of strain I6628^T was observed at temperatures between 40 and 73 °C; optimal growth occurred at 68 °C (see Supplementary Fig. S1, available in IJSEM Online). The doubling time and mean cell density reached at 68 °C were about 95 min and 1.85×10^8 cells ml⁻¹. To determine the effect of pH on growth, the pH of the medium was adjusted by altering the level of CO₂ saturation by changing the NaOH concentration and gassing the medium under different ratios of CO₂ and N₂ (Chong *et al.*, 2002). Sulfuric acid was used for pH values below

6.2. For pH values above 7.3, the MS medium was further modified by decreasing the amounts of $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$ (0.2 g l^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.4 g l^{-1}) and eliminating $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ from the medium. This was done to eliminate precipitates and did not appear to affect growth, as growth rates were consistent with those obtained at lower pH (data not shown). Growth of the isolate at 68°C was observed between pH 5.3 and 7.8, with optimal growth at pH 6.6 (see Supplementary Fig. S2, available in IJSEM Online). The doubling time and mean cell density at pH 6.6 and 68°C were about 100 min and $4.0 \times 10^8 \text{ cells ml}^{-1}$. Salt tolerance was tested by injecting defined amounts of NaCl (0–3 %, w/v) into MS medium (pH 6) for incubation in a water bath at 68°C . Optimal growth was observed in the absence of NaCl, but growth occurred with NaCl at concentrations up to 0.5 %, w/v (data not shown).

Strain I6628^T was tested for the ability to oxidize a variety of inorganic electron donors with O_2 (4 %, v/v) or nitrate (as NaNO_3 ; 0.1 %, w/v) serving as the terminal electron acceptors. Donors tested included H_2 (145 kPa), S^0 (3 %, w/v), thiosulfate (as $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$; 0.1 %, w/v), sulfite (as NaSO_3 ; 0.1 %, w/v), arsenite (as NaAsO_2 ; 5 mM), selenite (as Na_2SeO_3 ; 5 mM) and Fe^{2+} (as $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$; 5 mM). Growth was observed only with H_2 , S^0 and $\text{S}_2\text{O}_3^{2-}$ in the presence of O_2 . A variety of inorganic electron acceptors were also tested with H_2 (145 kPa) as the electron donor, and included S^0 (3 %, w/v), O_2 (1–25 %, v/v), thiosulfate (0.1 %, w/v), sulfite (0.1 %, w/v), nitrate (as NaNO_3 ; 0.1 %, w/v), nitrite (as NaNO_2 ; 0.01 and 0.1 %, w/v), Fe^{3+} (as ferric citrate; 5 mM), arsenate (as $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$; 5 mM) and selenate (as Na_2SeO_4 ; 5 mM). Strain I6628^T was able to utilize only O_2 (up to 25 %) as a terminal electron acceptor, showing optimal growth between 4 and 9 % (data not shown).

To test the novel isolate for heterotrophic and fermentative growth, a variety of organic carbon sources were added to modified MS medium in the absence of CO_2 , S^0 and $\text{S}_2\text{O}_3^{2-}$. Growth was monitored in the presence and absence of both O_2 as an electron acceptor and H_2 as an electron donor. Potential energy substrates and carbon sources were added at 0.1 and 0.01 % (w/v; v/v for liquids) and included yeast extract, Bacto peptone, trypticase peptone, sucrose, glucose, starch, sodium formate, Casamino acids, formaldehyde, formamide, sodium citrate, sodium propionate, sodium acetate, 2-propanol, mannose, succinate and oxalate (Aguiar *et al.*, 2004). Cultures that exhibited growth were transferred at least twice to ensure that growth was not the result of carry-over from the initial transfer. Strain I6628^T was only able to utilize succinate as a carbon source with H_2 as an electron donor and O_2 as an acceptor. The results of all of the growth experiments and comparisons with other *Sulfurihydrogenibium* isolates are summarized in Table 1.

Genomic DNA used for sequencing and G+C content determination was extracted from a 1 l culture by using the

Qiagen Genomic-tip 100/G DNA extraction kit according to the manufacturer's protocol for bacterial culture preparations. The thermal denaturation method (Marmur & Doty, 1962) was used to determine the genomic G+C content of strain I6628^T. The DNA G+C content of strain I6628^T was found to be 28.1 mol%, which is the lowest value reported for any described *Sulfurihydrogenibium* species (Table 1).

Amplification of the 16S rRNA gene and subsequent purification and sequencing were conducted as described previously (Ferrera *et al.*, 2007). An almost-complete 16S rRNA gene sequence (1486 nt) was assembled using the software AUTOASSEMBLER and was compared, using BLAST, against the NCBI non-redundant database. The ARB program (<http://www.arb-home.de>; Ludwig *et al.*, 2004) was used to align the 16S rRNA gene sequences according to secondary structure constraints. A similarity matrix using 1430 nt was constructed using a subset of closely related *Sulfurihydrogenibium* species. Strain I6628^T is most closely related to the 16S rRNA gene sequence of an environmental isolate from Iceland, designated strain SRI-240 (99.5 % sequence similarity; Skirnisdottir *et al.*, 2000). Only unambiguous nucleotide positions were used in the phylogenetic analysis (~1300 bp). Neighbour-joining (1000 bootstrap replications) and maximum-likelihood (100 bootstrap replications) analyses (PAUP* 4.0 beta 10; Swofford, 2003) were performed as described previously (Ferrera *et al.*, 2007) (Fig. 2).

On the basis of the 16S rRNA gene sequence analysis, strain I6628^T forms a distinct clade within the *Sulfurihydrogenibium* group (99 % maximum-likelihood bootstrap value) with the sequence of an environmental clone (SRI-240; Skirnisdottir *et al.*, 2000) from an Icelandic hot spring, and represents a novel cultivated species within this genus. Of the species of *Sulfurihydrogenibium* isolated to date, the strain closest to I6628^T is *S. rodmanii* UZ3-5^T (98.2 % 16S rRNA gene sequence similarity) (Fig. 2), which was isolated from Kamchatka, Russia. Strain I6628^T shows 16S rRNA gene sequence similarities of 98, 97.5 and 96 % with respect to *S. azorensis* Az-Ful^T, *S. subterraneum* HG MK-1^T and *S. yellowstonense* SS-5^T (Fig. 2), respectively.

The members of the *Aquificales* appear to be the predominant primary producers in circumneutral terrestrial hot-spring streams at temperatures above the limits for photosynthesis (Reysenbach *et al.*, 2005; Spear *et al.*, 2005) and in conditions where hydrogen, sulfur/sulfide and oxygen are readily available. Micro-organisms belonging to the genus *Sulfurihydrogenibium* tend to be the predominant members of the *Aquificales* present in circumneutral terrestrial springs with elevated sulfide concentrations. This situation contrasts with that found in low-sulfide springs, in which other members of the *Aquificales* that preferentially oxidize hydrogen, e.g. *Thermocrinis* and *Hydrogenobacter*, predominate (Skirnisdottir *et al.*, 2000); both *Thermocrinis* and *Hydrogenobacter* can also oxidize reduced sulfur compounds (Huber *et al.*, 1998; Stöhr *et al.*,

Table 1. Comparison of physiological traits of strain I6628^T and described members of the genus *Sulfurihydrogenibium*

Characteristic	Strain I6628 ^T	<i>S. rodmanii</i> UZ3-5 ^T	<i>S. yellowstonense</i> SS-5 ^T	<i>S. azorensis</i> Az-Ful ^T	<i>S. subterraneum</i> HGMK-1 ^T
Origin	Terrestrial hot spring, Iceland	Terrestrial hot spring, Kamchatka, Russia	Terrestrial hot spring, Yellowstone National Park, USA	Terrestrial hot spring, the Azores, Portugal	Subsurface gold mine, Hishikari, Japan
Cell size (µm) (length × width)	1.5–2.5 × 0.4–0.5	1.19–1.42 × 0.72–0.79	1.2–2.8 × 0.6–0.8	0.9–2.0 × 0.4–0.5	1.5–2.5 × 0.3–0.5
Temperature range (optimum) (°C)	40–73 (68)	55–80 (74–75)	55–78 (70)	50–73 (68)	40–70 (65)
pH range (optimum)	5.3–7.8 (6.6)	5.0–7.0 (5.5–6.47)	6.0–8.0 (7.5)	5.5–7.0 (6.0)	6.4–8.8 (7.5)
NaCl range (optimum) (% v/w)	0–0.5 (0)	0–0.9 (0)	0–0.6 (0)	0–0.25 (0.1)	0–4.8 (0.5)
Electron donors	H ₂ , S ⁰ , S ₂ O ₃ ²⁻	S ⁰ , S ₂ O ₃ ²⁻	S ⁰ , S ₂ O ₃ ²⁻	H ₂ , S ⁰ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻ , Fe ²⁺ , * AsO ₃ ³⁻ *	H ₂ , S ⁰ , S ₂ O ₃ ²⁻
Electron acceptor(s)	O ₂ (up to 25 %, v/v)	O ₂ (up to 14 %, v/v)	O ₂ (up to 18 %, v/v)	S ⁰ , Fe ³⁺ , * O ₂ (up to 9 %, v/v), SO ₃ ²⁻ , AsO ₄ ³⁻ , * SeO ₄ ²⁻ *	Fe ³⁺ , O ₂ , NO ₃ ⁻ , HAsO ₄ ²⁻ , SeO ₄ ²⁻ , SeO ₃ ²⁻
Organic carbon source(s)	Succinate (with H ₂ as electron donor)	None	Yeast extract, Bacto peptone, trypticase peptone, sucrose, glucose, starch, formate, Casamino acids, citrate, propionate, acetate	Yeast extract, Bacto peptone, trypticase peptone, Casamino acids	Acetate
Internal structures	Stacked membranes/fibres	Not detected	Not detected	Stacked membranes/fibres	No internal structures reported
DNA G + C content (mol%)	28.1	35	32	33.6	31.3

*We have recently had difficulty growing this organism under these conditions.

Fig. 2. Maximum-likelihood phylogenetic tree, based on 16S rRNA gene sequences, showing the position of strain I6628^T relative to members of the Aquificales. Bootstrap percentages above 50 % are shown firstly for the neighbour-joining analyses (based on 1000 replicates) and secondly for the maximum-likelihood analysis (based on 100 replicates). Additional sequences used to generate the tree (not shown) were from *Aquifex pyrophilus* Kol5a^T (GenBank accession no. M83548), *Hydrogenobacter subterraneus* HGP1^T (AB026268), *Thermocrinis ruber* OC 1/4^T (AJ005640), *Persephonella marina* EX-H1^T (AF188332), *Persephonella hydrogeniphila* 29W^T (AB086419), *Desulfurobacterium thermolithotrophum* BSA^T (AJ001049) and *Thermotoga maritima* MSB8^T (M21774). The sequence of *Methanocaldococcus jannaschii* DSM 2661^T (GenBank accession no. M59126) was used as the outgroup to root the tree (not shown). Bar, 10 substitutions per 100 nucleotides.

2001; Eder & Huber 2002). However, it appears that *Sulfurihydrogenibium* species might be more adapted to high-sulfide environments and are able to outcompete other members of the Aquificales (Skirnisdottir *et al.*, 2000). Furthermore, some isolates, e.g. *S. yellowstonense* SS-5^T, lack the suite of hydrogenases present in '*Aquifex aeolicus*' VF5 and *S. azorensis* Az-Fu1^T (A.-L. Reysenbach, unpublished results). The isolation of strain I6628^T under sulfur-oxidizing conditions serves to illustrate the importance of sulfide to the physiological ecology of the genus *Sulfurihydrogenibium*.

Strain I6628^T shares several of the physiological properties associated with recognized species of the genus *Sulfurihydrogenibium*, including the preference for low concentrations of NaCl and O₂ and the ability to oxidize S⁰ and S₂O₃²⁻ (Table 1). In contrast, only three of the described isolates, strain I6628^T, *S. azorensis* Az-Fu1^T (Aguiar *et al.*, 2004) and *S. subterraneum* HGMK-1^T (Takai *et al.*, 2003), can oxidize H₂. Additionally, only I6628^T, *S. rodmanii* UZ3-5^T (O'Neill *et al.*, 2008) and *S. yellowstonense* SS-5^T (Nakagawa *et al.*, 2005) use only O₂ as an electron acceptor. Strain I6628^T also possesses heterotrophic capabilities similar to those of *S. azorensis* Az-Fu1^T, *S. subterraneum* HGMK-1^T and *S. yellowstonense* SS-5^T, but is much more restricted, using only succinate as an organic carbon source. Therefore, it appears that strain I6628^T possesses a combination of the physiological capabilities of all *Sulfurihydrogenibium* species described to date. On the basis of these phylogenetic and physiological characteristics, strain I6628^T represents a novel species of the genus *Sulfurihydrogenibium*, for which the name *Sulfurihydrogenibium kristjanssonii* sp. nov. is proposed.

Description of *Sulfurihydrogenibium kristjanssonii* sp. nov.

Sulfurihydrogenibium kristjanssonii (krist.jans'son.i.i. N.L. masc. gen. n. *kristjanssonii* of Kristjansson, in honour of Jakob Kristjansson for his long-term commitment to the description and exploration of thermophile biodiversity in Iceland, which includes some of the first work done on the Aquificales).

Cells are motile, Gram-negative, straight to slightly curved rods with mean lengths of 1.5–2.5 µm and widths of approximately 0.5 µm. Occur singly or in filaments consisting of a few cells. Grows between 40 and 73 °C (optimally at 68 °C), between pH 5.3 and 7.8 (optimally at pH 6.6) and at NaCl concentrations between 0 and 0.5 % (w/v). Grows with H₂, S⁰ and S₂O₃²⁻ as electron donors and O₂ as sole electron acceptor (up to 25 %, v/v). Facultatively heterotrophic, being capable of using succinate and CO₂ as carbon sources. The G + C content of the genomic DNA of the type strain is 28.1 mol%.

The type strain, I6628^T (=DSM 19534^T =OCM 901^T =ATCC BAA-1535^T), was isolated from the outflow channel of a hot spring near the town of Hveragerdi, Iceland.

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References

- Aguilar, P., Beveridge, T. J. & Reysenbach, A.-L. (2004). *Sulfurihydrogenibium azorense* sp. nov., a thermophilic hydrogen-oxidizing microaerophile from terrestrial hot springs in the Azores. *Int J Syst Evol Microbiol* **54**, 33–39.
- Barion, S., Franchi, M., Gallori, E. & Di Giulio, M. (2007). The first lines of divergence in the Bacteria domain were the hyperthermophilic organisms, the *Thermotogales* and the *Aquificales*, and not the mesophilic *Planctomycetales*. *Biosystems* **87**, 13–19.
- Beveridge, T. J., Moyses, D. & Harris, R. (2007). Electron microscopy. In *Methods for General and Molecular Microbiology*, 2nd edn, pp. 54–81. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, L. Snyder, T. M. Schmidt & G. A. Marzluf. Washington, DC: American Society for Microbiology.
- Boone, D. R., Johnson, R. L. & Liu, Y. (1989). Diffusion of the interspecies electron carriers H_2 and formate in methanogenic ecosystems and its implications in the measurement of K_m for H_2 or formate uptake. *Appl Environ Microbiol* **55**, 1735–1741.
- Burggraf, S., Olsen, G. J., Stetter, K. O. & Woese, C. R. (1992). A phylogenetic analysis of *Aquifex pyrophilus*. *Syst Appl Microbiol* **15**, 352–356.
- Chong, S. C., Liu, Y., Cummins, M., Valentine, D. L. & Boone, D. R. (2002). *Methanogenium marinum* sp. nov., a H_2 -using methanogen from Skan Bay, Alaska, and kinetics of H_2 utilization. *Antonie van Leeuwenhoek* **81**, 263–270.
- Di Giulio, M. (2003a). The ancestor of the Bacteria domain was a hyperthermophile. *J Theor Biol* **224**, 277–283.
- Di Giulio, M. (2003b). The universal ancestor and the ancestor of the Bacteria were hyperthermophiles. *J Mol Evol* **57**, 721–730.
- Di Giulio, M. (2003c). The universal ancestor was a thermophile or a hyperthermophile: tests and further evidence. *J Theor Biol* **221**, 425–436.
- Eder, W. & Huber, R. (2002). New isolates and physiological properties of the *Aquificales* and description of *Thermocrinis albus* sp. nov. *Extremophiles* **6**, 309–318.
- Ferguson, T. J. & Mah, R. A. (1983). Isolation and characterization of an H_2 -oxidizing methanogen. *Appl Environ Microbiol* **45**, 265–274.
- Ferrera, I., Longhorn, S., Banta, A. B., Liu, Y., Preston, D. & Reysenbach, A.-L. (2007). Diversity of 16S rRNA gene, ITS region and *aclB* gene of the *Aquificales*. *Extremophiles* **11**, 57–64.
- 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.
- Hetzer, A., Morgan, H. W., McDonald, I. R. & Daughney, C. J. (2007). Microbial life in Champagne Pool, a geothermal spring in Waiotapu, New Zealand. *Extremophiles* **11**, 605–614.
- Huber, R., Eder, W., Heldwein, S., Wanner, G., Huber, H., Rachel, R. & Stetter, K. O. (1998). *Thermocrinis ruber* gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park. *Appl Environ Microbiol* **64**, 3576–3583.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Nakagawa, S., Shtaih, Z., Banta, A., Beveridge, T. J., Sako, Y. & Reysenbach, A.-L. (2005). *Sulfurihydrogenibium yellowstonense* sp. nov., an extremely thermophilic, facultatively heterotrophic, sulfur-oxidizing bacterium from Yellowstone National Park, and emended descriptions of the genus *Sulfurihydrogenibium*, *Sulfurihydrogenibium subterraneum* and *Sulfurihydrogenibium azorense*. *Int J Syst Evol Microbiol* **55**, 2263–2268.
- O'Neill, A. H., Liu, Y., Ferrera, I., Beveridge, T. J. & Reysenbach, A.-L. (2008). *Sulfurihydrogenibium rodmanii* sp. nov., a sulfur-oxidizing chemolithoautotroph from the Uzon Caldera, Kamchatka, Russia, and emended description of the genus *Sulfurihydrogenibium*. *Int J Syst Evol Microbiol* **58**, 1147–1152.
- Pitulle, C., Yang, Y. Q., Marchiani, M., Moore, E. R. B., Siefert, J. L., Aragno, M., Jurtshuk, P. & Fox, G. E. (1994). Phylogenetic position of the genus *Hydrogenobacter*. *Int J Syst Bacteriol* **44**, 620–626.
- Purcell, D., Sompong, U., Yim, L. C., Barraclough, T. G., Peerapornpisal, Y. & Pointing, S. B. (2007). The effects of temperature, pH and sulphide on the community structure of hyperthermophilic streamers in hot springs of northern Thailand. *FEMS Microbiol Ecol* **60**, 456–466.
- Reysenbach, A.-L., Ehringer, M. & Hershberger, K. (2000). Microbial diversity at 83°C in Calcite Springs, Yellowstone National Park: another environment where the *Aquificales* and “Korarchaeota” coexist. *Extremophiles* **4**, 61–67.
- Reysenbach, A.-L., Banta, A., Civello, S., Daly, J., Mitchel, K., Lalonde, S., Konhauser, K., Rodman, S., Rustenholtz, K. & Takacs-Vesbach, C. (2005). The *Aquificales* of Yellowstone National Park. In *Geothermal Biology and Geochemistry in Yellowstone National Park: Workshop Proceedings from the Thermal Biology Institute's Yellowstone National Park Conference, October 2003*, pp. 129–142. Edited by W. P. Inskeep & T. R. McDermott. Bozeman, MT: Montana State University.
- Skirnisdottir, S., Hreggvidsson, G. O., Hjorleifsdottir, S., Marteinson, V. T., Petursdottir, S. K., Holst, O. & Kristjansson, J. K. (2000). Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats. *Appl Environ Microbiol* **66**, 2835–2841.
- Spear, J. R., Walker, J. J., McCollom, T. M. & Pace, N. R. (2005). Hydrogen and bioenergetics in the Yellowstone geothermal ecosystem. *Proc Natl Acad Sci U S A* **102**, 2555–2560.
- Stöhr, R., Waberski, A., Völker, H., Tindall, B. J. & Thomm, M. (2001). *Hydrogenothermus marinus* gen. nov., sp. nov., a novel thermophilic hydrogen-oxidizing bacterium, recognition of *Calderobacterium hydrogenophilum* as a member of the genus *Hydrogenobacter* and proposal of the reclassification of *Hydrogenobacter acidophilus* as *Hydrogenobaculum acidophilum* gen. nov., comb. nov., in the phylum ‘*Hydrogenobacter/Aquifex*’. *Int J Syst Evol Microbiol* **51**, 1853–1862.
- Swofford, D. L. (2003). PAUP*: Phylogenetic analysis using parsimony (and other methods). Sunderland, MA: Sinauer Associates.
- Takacs, C. D., Ehringer, M., Favre, R., Cermola, M., Eggertsson, G., Palsdottir, A. & Reysenbach, A.-L. (2001). Phylogenetic characterization of the blue filamentous bacterial community from an Icelandic geothermal spring. *FEMS Microbiol Ecol* **35**, 123–128.
- Takai, K., Kobayashi, Y., Nealson, K. H. & Horikoshi, K. (2003). *Sulfurihydrogenibium subterraneum* gen. nov., sp. nov., from a subsurface hot aquifer. *Int J Syst Evol Microbiol* **53**, 823–827.