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ORIGINAL PAPER

Wolfgang Eder · Robert Huber

New isolates and physiological properties of the *Aquificales* and description of *Thermocrinis albus* sp. nov.

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Abstract The ecology of the *Aquificales* was studied using a combination of phylogenetic and cultivation approaches. Enrichment cultures were prepared from low-salt and marine samples of geothermally and volcanically heated environments of the United States (Yellowstone National Park), Russia (Kamchatka), Italy, Germany, Djibouti, Iceland, and Africa (Lake Tanganyika). Isolation of single cells using the selected cell cultivation technique resulted in 15 different pure cultures. Comparisons of their 16S rRNA gene sequences showed that most of the isolates were new representatives of the major lineages of the *Aquificaceae*, represented by the genera *Aquifex*, *Thermocrinis*, *Hydrogenobaculum*, and *Hydrogenobacter*. Isolate HI 11/12, which was obtained from whitish streamers in the Hveragerthi area of Iceland, represents a separate branch within the *Aquificaceae*. The organism grew at salinities up to 0.7% NaCl and at temperatures up to 89°C. Depending on the culture conditions, the organisms occurred as single motile rods, as aggregates, or as long filaments that formed whitish streamer-like cell masses. The novel isolate grew chemolithoautotrophically with hydrogen, sulfur, or thiosulfate as the electron donor under microaerophilic conditions. It represents a second species within the order *Thermocrinis*, which we name *Thermocrinis albus* HI 11/12 (DSM 14484, JCM 11386).

Key words High-temperature ecosystems · *Aquificales* · *Thermocrinis* · *Aquifex* · *Hydrogenobacter* · 16S rRNA gene sequences · Selected cell cultivation

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W. Eder · R. Huber (✉)
Lehrstuhl für Mikrobiologie und Archaeenzentrum, Universität Regensburg, Universitätsstr. 31, D-93053 Regensburg, Germany
Tel. +49-941-9433182; Fax +49-941-9432403
e-mail: robert.huber@biologie.uni-regensburg.de

Introduction

On the basis of 16S rRNA gene sequence comparisons, the order *Aquificales* represents a very deep phylogenetic branch within the bacterial domain (Burggraf et al. 1992). To date, four genera, *Aquifex*, *Thermocrinis*, *Hydrogenobacter*, and *Hydrogenobaculum*, have been identified as belonging to the *Aquificaceae* within the *Aquificales* (Pitulle et al. 1994; Huber et al. 1998; Stöhr et al. 2001; Takai et al. 2001; Huber and Stetter, Encyclopedia of Life Sciences: *Aquificales* http://www.els.net). Very recently, *Hydrogenobacter acidophilus*, which has been reclassified as *Hydrogenobaculum acidophilum*, was determined to form a separate phylogenetic branch within the *Aquificales* (Shima et al. 1994; Stöhr et al. 2001). Moreover, the lineage of the *Aquificales* contains related environmental sequences from diverse hydrothermal systems on earth (Hugenholtz et al. 1998; Yamamoto et al. 1998; Reysenbach et al. 2000b, c; Skirnisdottir et al. 2000; Marteinsson et al. 2001; Takacs et al. 2001). The first described organism of this group is *Hydrogenothermus marinus* (Stöhr et al. 2001).

Cultivated representatives of the *Aquificales* have been obtained mostly from water-containing, volcanically and geothermally heated natural environments situated mainly along terrestrial and submarine tectonic fracture zones. Whereas members of the genus *Aquifex* have been obtained exclusively from marine biotopes (e.g., Kolbeinsey Ridge, north of Iceland), members of the genera *Thermocrinis*, *Hydrogenobacter*, and *Hydrogenobaculum* have been found mainly in terrestrial environments (e.g., Yellowstone National Park, USA; Geyser Valley, Kamchatka; Huber and Stetter, Encyclopedia of Life Sciences: *Aquificales* http://www.els.net). In addition, some *Hydrogenobacter* relatives have been obtained from hot compost and the deep sea (Beffa et al. 1996; Takai et al. 2001).

Members of the *Aquificales* are extremely thermophilic or hyperthermophilic bacteria; some have an upper temperature limit of growth of 95°C (Huber and Stetter, Encyclopedia of Life Sciences: *Aquificales* http://www.els.net). Together with members of the order *Thermotogales*, they represent the bacteria with the highest

growth temperatures known so far (Huber and Stetter, Encyclopedia of Life Sciences: *Thermotogales* http://www.els.net). The *Aquificales* exhibit a rod-shaped morphology and show a negative Gram reaction. Within a permanent flow of medium, long filaments are formed by *Thermocrinis ruber* (Huber et al. 1998, 2001). Optimal growth of the *Aquificales* can be obtained in the neutral pH range (except *Hb. acidophilum*; Shima and Suzuki 1993; Stöhr et al. 2001; Takai et al. 2001; Huber and Stetter, Encyclopedia of Life Sciences: *Aquificales* http://www.els.net). Almost all of them are able to grow chemolithoautotrophically with hydrogen, thiosulfate, or sulfur as electron donor and oxygen as electron acceptor (Kryukov et al. 1983; Kawasumi et al. 1984; Kristjansson et al. 1985; Bonjour and Aragno 1986; Shima and Suzuki 1993; Huber et al. 1992, 1998; Stöhr et al. 2001). Nitrate is used by *Aquifex pyrophilus* and *Hydrogenobacter thermophilus* TK-6 as an alternative electron acceptor (Huber et al. 1992; Suzuki et al. 2001). In the presence of sulfur, the *Aquifex* species and *T. ruber* produce hydrogen sulfide in addition to sulfuric acid. In contrast to *Aquifex* and *Hydrogenobacter*, *T. ruber* shows a metabolic flexibility, growing also chemo-organoheterotrophically on formate or formamide (Huber et al. 1998).

The aim of the present study was to evaluate the ecology of the *Aquificales* using a combination of phylogenetic and cultivation methods. The phylogenetic diversity of the new isolates was investigated using 16S rRNA gene sequence comparisons, and cultivation and isolation were prerequisite for the elucidation of metabolic and biochemical properties within the *Aquificales*.

Materials and methods

Sources of strains

16S rRNA gene sequence information was determined from 15 *Aquificales* members and compared with the 16S rRNA gene sequences of the published *Aquificales* (Table 1). *Aquifex pyrophilus* DSM 6858 and *Thermocrinis ruber*

Table 1. Sources of *Aquificales* and their 16S rRNA gene sequences

Organism	Isolated from	Accession no.
Aquifex aeolicus VF5	Hydrothermal system, Porto di Levante, Vulcano, Italy (102°C)	AJ309733
Aquifex pyrophilus Kol5a (DSM 6858) [T][a]	Hot vent, Kolbeinsey Ridge, north of Iceland (depth: 106 m)	M83548
Aquifex sp. Gri14L3B	Anhydride smoker, Grimsey hydrothermal area, north of Iceland (100°–110°C, pH 7.5, 403 m)	AJ320223
Aquifex sp. Ob6	Coastal hot spring, Obock, Gulf of Tadjoura, Djibouti	AJ320224
Hydrogenobacter hydrogenophilus Z-829 (DSM 2913)[a]	Hot spring, Geyser Valley, Kamchatka, Russia (56°C)	Z30242
Hydrogenobacter subterraneus HGP1 (JCM 10560)[a]	Hacchoubaru, geothermal plant, Oita Prefecture, Japan	AB026268
Hydrogenobacter thermophilus TK-6 (IAM 12695) [T][a]	Hot spring, Mine, Izu, Japan	Z30214
Hydrogenobacter thermophilus T3[a]	S. Federigo solfatara, Province de Grosetto, Italy (98°C, pH 6.5)	Z30189
Hydrogenobacter sp. BB4L1B	Kirchenstollen, Baden-Baden, Germany (61°C, pH 6.8)	AJ320216
Hydrogenobacter sp. GV8L3A	Hot streamlet, Geyser Valley, Kamchatka, Russia (70°–80°C)	AJ320218
Hydrogenobacter sp. MV4L2B	Hot spring, Mutnovski volcano, Kamchatka, Russia (80°C, pH 5.8)	AJ320214
Hydrogenobacter sp. PA14	Hot vent, Pemba, Lake Tanganyika (88°C, depth: 24 m)	AJ320215
Hydrogenobacter sp. PZ2AL1B	Mud hole, Pozzuoli, Italy (97°C, pH 6.5)	AJ320213
Hydrogenobacter sp. UZ27L2A	Hot spring, Uzon Valley, Kamchatka, Russia (80°–84°C, pH 5)	AJ320217
Hydrogenobaculum acidophilum 3H-1 JCM 8795 [T][a]	Solfatara mud sample, Tsumagoi, Gunma, Japan	D16296
Hydrogenobaculum sp. NOR3L3B	Mud hole, Norris Geyser Basin, Yellowstone National Park, Wyoming, USA (91°C, pH 3)	AJ320225
Hydrogenothermus marinus VM1 (DSM 12046) [T][a]	Hydrothermal system, beach of Vulcano, Italy	AJ292525
Thermocrinis ruber OC1/4 (DSM 12173) [T][a]	"Pink filaments", upper outflow channel of Octopus Spring, White Creek Area, Lower Geyser Basin, Yellowstone National Park, Wyoming, USA (82°–88°C, pH 8)	AJ005640
Thermocrinis albus HI 11/12	Grayish filaments, hot streamlet, Hveragerthi, Iceland (88°C, pH 7.5)	AJ278895
Thermocrinis sp. G3L1B	Whitish filaments, hot spring, Graendalur, Iceland (72°C, pH 6)	AJ320221
Thermocrinis sp. H7L1B	Mud hole, Hveragerthi, Iceland (96°C, pH 7)	AJ320222
Thermocrinis sp. P2L2B	Lake View Terraces, Fissure Group, Heart Lake Geyser Basin, Yellowstone National Park, Wyoming, USA (65°C, pH 7)	AJ320219
Thermocrinis sp. UZ23L3A	Grayish filaments, mud hole, Uzon Valley, Kamchatka, Russia (80°C, pH 5)	AJ320220

[T], type species; DSM = DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; IAM, culture collection of the Institute of Applied Microbiology, University of Tokyo, Tokyo, Japan; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan
[a] Previously published

DSM 12173 were from the culture collection of our institute. *Hydrogenobacter thermophilus* IAM 12695 was obtained from the culture collection of the Institute of Applied Microbiology, University of Tokyo.

Sampling, cultivation, and isolation of new strains

Sources of the *Aquificales*, including the new strains, and their accession numbers are given in Table 1. The new *Aquificales* strains were enriched, isolated, and cultivated using the media indicated in Table 2. The majority of the new isolates were obtained by the use of "optical tweezers" (selected cell cultivation; Huber et al. 1995, 2000; Beck and Huber 1997). Cell masses of *Aquifex pyrophilus*, *Aquifex aeolicus*, *Hydrogenobacter thermophilus* TK-6, *Thermocrinis ruber*, and isolate HI 11/12 were grown at 80°C (70°C for *H. thermophilus*) with stirring (up to 41.9 rad/s) in a 300-l enamel-protected fermenter (HTE Bioengineering, Wald, Switzerland), as described elsewhere (Jahnke et al. 2001). Growth studies of isolate HI 11/12 under a permanent flow of medium (OS-medium + 0.1% thiosulfate, wt/vol; pH 6.5) were carried out in a glass chamber under contact with air at a temperature of 80°–85°C as described for *T. ruber* (Huber et al. 1998). The flow rate was 15 ml/min.

Light and electron microscopy

Light microscopy, transmission and scanning electron microscopy, energy dispersive X-ray microanalysis, and photography were carried out as described elsewhere (Huber et al. 1998).

Test for diaminopimelic acid and lipid analysis

Diaminopimelic acid was assayed by thin-layer chromatography (Rhuland et al. 1955). Lipid extraction, separation, and analysis were carried out as described previously (Jahnke et al. 2001).

DNA extraction, PCR, and sequencing

Nucleic acids of the new strains were extracted from 20 to 40 ml culture by the freeze-thaw lysis procedure of Barns et al. (1994). The mixture was extracted with an equal volume of phenol (pH 7.5; AquaPhenol, Appligene, Illkirch, France), followed by extraction with phenol–chloroform–isoamyl alcohol, 24 : 24 : 1 (by volume), by using Eppendorf Phase Lock Gel Light (Hamburg, Germany). Nucleic acids were precipitated with 0.1 volume of 3 M (wt/vol) sodium acetate (pH 5.2) and 0.6 volume of isopropanol, washed with 70% (wt/vol) ethanol, and dried. In addition, an RNase treatment (2–5 µg RNase/ml) was performed. PCR amplification of the rRNA genes between *Escherichia coli* positions (Brosius et al. 1981) 9 and 1,512 were carried out as described previously (Table 3; Eder et al. 1999). The corresponding PCR products were purified with Microcon 100 (Amicon, Witten, Germany) and sequenced with an ABI Prism 310 capillary DNA Sequencer (PE Applied Biosystems, Foster City, CA, USA) by using the ABI Prism BigDye terminator cycle sequencing ready reaction kit (Perkin Elmer) at the Institute for Genetics, University of Regensburg, Germany. The 16S rRNA gene sequences were determined using a set of specific and universal primers (Table 3).

Table 2. Culture conditions of *Aquificales* isolates studied

Organism	Medium (wt/vol)	Gas phase (by volume)	Temperature (°C)	pH	Reference
Aquifex aeolicus VF5	SME*-medium + 0.1% thiosulfate	H_2-CO_2-O_2 (79 : 20 : 1)	85	6.5	Huber et al. 1992; Huber and Stetter 2001
Aquifex pyrophilus Kol5a	SME*-medium	H_2-CO_2-O_2 (79 : 20 : 1)	85	6.5	Huber et al. 1992
Aquifex sp. Gri14L3B	1/2 SME, modified	H_2-CO_2-O_2 (79 : 20 : 1)	85	5.5	Huber et al. 1990
Aquifex sp. Ob6	SME*-medium	H_2-CO_2-O_2 (79 : 20 : 1)	75	7	Huber et al. 1992; this study
Hydrogenobacter thermophilus TK-6	TK6-medium	H_2-CO_2-O_2 (79 : 20 : 1)	70	7	Kawasumi et al. 1984; Ishii et al. 1987
Hydrogenobacter sp. BB4L1B	TK6-medium + 0.2% thiosulfate	H_2-CO_2-O_2 (79 : 20 : 1)	75	7	Ishii et al. 1987; this study
Hydrogenobacter sp. GV8L3A	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	85	6	Huber et al. 1998; this study
Hydrogenobacter sp. MV4L2B	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	85	6	Huber et al. 1998; this study
Hydrogenobacter sp. PA14	TK6-medium	H_2-CO_2-O_2 (79 : 20 : 1)	80	7	Ishii et al. 1987; this study
Hydrogenobacter sp. PZ2AL1B	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	85	6	Huber et al. 1998; this study
Hydrogenobacter sp. UZ27L2A	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	80	6	Huber et al. 1998; this study
Hydrogenobaculum sp. NOR3L3B	Allen-medium	H_2-CO_2-O_2 (79 : 20 : 1)	65	3	Allen 1959; this study
Thermocrinis ruber OC1/4	OS-medium + 0.1% thiosulfate	N_2-H_2-O_2 (94 : 3 : 3)	80	7	Huber et al. 1998
Thermocrinis albus HI 11/12	OS-medium + 0.1% thiosulfate	N_2-H_2-O_2 (96 : 3 : 1)	85	7	Huber et al. 1998; this study
Thermocrinis sp. G3L1B	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	85	7	Huber et al. 1998; this study
Thermocrinis sp. H7L1B	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	80	7	Huber et al. 1998; this study
Thermocrinis sp. P2L2B	OS-medium + 0.1% thiosulfate	N_2-H_2-O_2 (96 : 3 : 1)	85	7	Huber et al. 1998; this study
Thermocrinis sp. UZ23L3A	OS-medium + 0.05% sulfur	N_2-H_2-O_2 (96 : 3 : 1)	85	6	Huber et al. 1998; this study

Table 3. Oligonucleotide primers used for PCR amplification and sequencing reactions of the 16S rRNA genes. The target sites are based on the *E. coli* sequence numbering according to Brosius et al. (1981)

Oligonucleotide	Target site	Sequence (5′→3′)	Specificity	Reference
9bF	9–27	GRGTTTGATCCTGGCTCAG	Bacteria	Burggraf et al. 1992
519uF	519–536	CAGCMGCCGCGGTAATAC	Universal	Eder et al. 1999
704bR	687–704	TCTACGYATTTCACYGCT	Bacteria	Stetter and Burggraf, unpublished
1116bR	1,100–1,116	AGGGGTTGCGCTCGTTG	Bacteria	Stetter and Burggraf, unpublished
1406uR	1,390–1,406	ACGGGCGGTGTGTRCAA	Universal	Lane 1991
1512uR	1,492–1,512	ACGGHTACCTTGTTACGACTT	Universal	Lane 1991

Phylogenetic analyses

For the phylogenetic analyses, an alignment of about 11,000 homologous full (and partial) primary sequences available in public databases (ARB project, http://www.arb-home.de; Ludwig 1995) was used. The new 16S rRNA gene sequences (>1,357 nucleotides) were fitted in the 16S rRNA tree by using the respective automated tools of the ARB software package (http://www.arb-home.de). Distance matrix (Jukes and Cantor correction), maximum parsimony, and maximum likelihood (fastDNAml) methods were applied as implemented in the ARB software package (Ludwig et al. 1998; Page and Holmes 1998). Insignificant branching points were shown by multifurcation. Phylogenetic distances were determined by using a distance matrix without applying a correction factor. Each sequence alignment was checked manually, and the sequences were submitted to the CHECK_CHIMERA program of the Ribosomal Database Project (RDP) (Maidak et al. 2000) to detect the presence of possible chimeric artifacts.

DNA base composition

The G + C content of isolate HI 11/12 was determined by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany).

Nucleotide sequence accession numbers

The European Molecular Biology Laboratory (EMBL) accession numbers for the 16S rRNA gene sequences of the new *Aquificales* strains are AJ320213 to AJ320225, AJ309733 (*Aquifex aeolicus*), and AJ278895 (isolate HI 11/12).

Results

16S rRNA sequence analysis

For the phylogenetic analysis of the *Aquificales*, we determined nearly complete 16S rDNA sequences for the new isolates. The *Aquificales*, including the environmental sequence cluster, represent a monophyletic lineage, branching deeply in the bacterial domain (Fig. 1, new isolates shown in boldface). Within the *Aquificaceae* and the environmental sequence cluster, the most distantly related species have 19.5% and 11.2% sequence dissimilarity, indicating that the *Aquificales* are very diverse (Fig. 1). All of the algorithms utilized to reconstruct the phylogenetic tree (distance matrix, maximum parsimony, and maximum likelihood) yielded the same tree topology (data not shown). According to the phylogenetic tree, the order *Aquificales* is composed of the *Aquificaceae* and an environmental sequence cluster (including *Hydrogenothermus marinus*, isolated from the coast of Vulcano, Italy, and isolate EX-H1, obtained from a deep-sea hydrothermal system; Reysenbach et al. 2000a; Stöhr et al. 2001), which represents a second family (Yamamoto et al. 1998; Hugenholtz et al. 1998; Reysenbach et al. 2000b, c; Skirnisdottir et al. 2000). As a new family name, we propose *Hydrogenothermaceae*, owing to the isolation of *Hydrogenothermus marinus* as the first validly published organism within this novel phylogenetic lineage.

In the *Aquificaceae*, five major branches were identified. The genus *Hydrogenobacter* is composed of two sequence clusters, which are represented by *Hydrogenobacter thermophilus* TK-6 and *Hydrogenobacter thermophilus* T3 (Fig. 1; Pitulle et al. 1994). Closely related species to *H. thermophilus* TK-6 were obtained from Kamchatka (isolates GV8L3A and UZ27L2A) and to *H. thermophilus* T3 from Germany, Italy, Kamchatka, and Lake Tanganyika (isolates BB4L1B, PZ2AL1B, MV4L2B, and PA14) as indicated in Fig. 1. The genus *Thermocrinis* is represented by *T. ruber* (Huber et al. 1998). Closely related sequences were obtained from hot springs in Yellowstone National Park (isolate P2L2B and environmental sequences OPS 5 and EM17; Fig. 1; Reysenbach et al. 1994; Hugenholtz et al. 1998). Recently, the novel isolate HI 11/12 (designated *Thermocrinis albus*, see below) was obtained from grayish filaments found in the Hveragerthi area, representing a separate lineage with closest relationship to *Thermocrinis* (5.1% phylogenetic distance to *T. ruber*; Huber et al. 1998). Additional relatives of isolate HI 11/12 were identified in hot springs in Iceland and Kamchatka (isolates G3L1B, H7L1B, and UZ23L3A and environmental sequences SRI-48 and pICE; Skirnisdottir et al. 2000; Takacs et al. 2001; Fig. 1). Within the genus *Aquifex*, new members were obtained from "anhydride smokers" of the Grimsey hydrothermal system (isolate Gri14L3B) and from a coastal hot

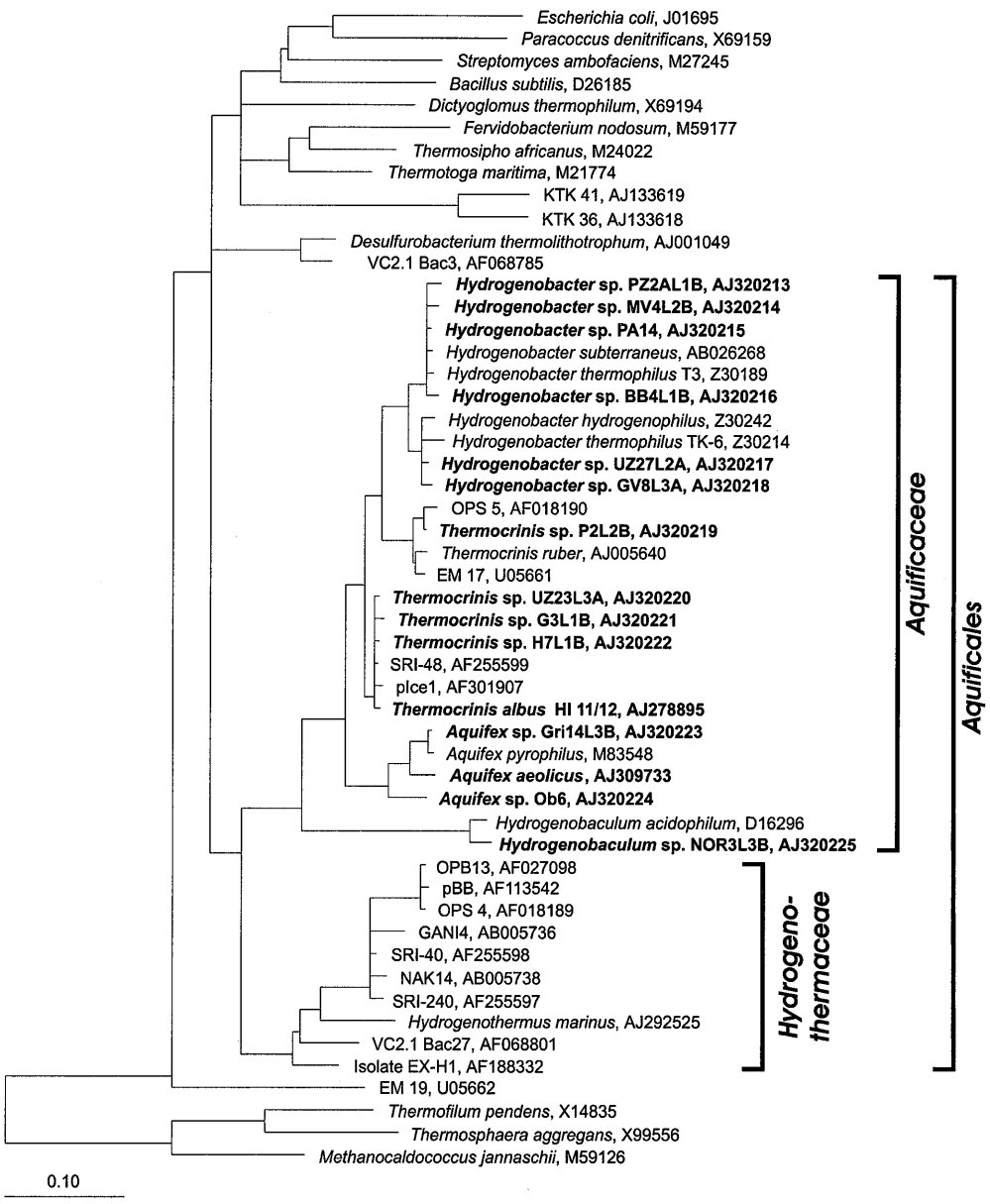

Fig. 1. 16S rRNA gene-based phylogenetic tree of the *Aquificales* including the 16S rRNA gene sequences of the new isolates (in *bold*) and the 16S rDNA sequences of environmental analyses obtained from public databases. The topology of the consensus tree is based on results of a maximum parsimony analysis. Reference sequences were chosen to represent the broadest diversity of *Bacteria*. Only sequence positions that share identical residues of 50% or more with the 16S rRNA sequences of the *Aquificales* were included for tree reconstruction. *Accession numbers* for the sequences are indicated. The *scale bar* represents 0.10 fixed mutations per nucleotide position

spring in Obock (isolate Ob6), Gulf of Tadjoura, Djibouti, as indicated in Fig. 1. *Hydrogenobaculum acidophilum* and the closely related isolate NOR3L3B from Yellowstone National Park, USA, comprise a separate lineage (Fig. 1).

Morphology

All new *Aquificales* isolates were rod-shaped cells with rounded ends and a Gram-negative stain reaction, consistent with the *Aquificales* representatives known to date (Huber and Stetter, Encyclopedia of Life Sciences: *Aquificales* http://www.els.net). Members of the genera *Aquifex*, *Hydrogenobacter*, *Calderobacterium*, and *Thermocrinis* grow singly or in pairs; *Aquifex* species and *Thermocrinis* can form flocs of up to 100 individual cells (Kryukov et al. 1983; Kawasumi et al. 1984; Kristjansson et al. 1985; Huber et al. 1992, 1998; Beffa et al. 1996). An unusual morphological feature of *Aquifex* species is the formation of wedge-shaped central or polar refractile areas during the logarithmic growth phase. Moreover, *Thermocrinis ruber* forms long filaments within a permanent water current, building up a pink network of cells (Huber et al. 1998). This growth behavior reflects the natural growth of the pink filaments in the rapidly flowing upper outflow channel of Octopus Spring in the White Creek area of the Lower Geyser Basin of Yellowstone National Park (Marler 1973; Brock 1978, 1995; Huber et al. 1998).

Similar to *T. ruber*, the new isolate HI 11/12 exhibits filamentous growth when cultivated under a permanent flow of medium in a glass chamber (see Materials and methods). Cell masses (attached to pieces of cotton) showed a white cell color after one day of incubation. Cells occurred singly, in pairs, and in aggregates (>100 cells per aggregate). After

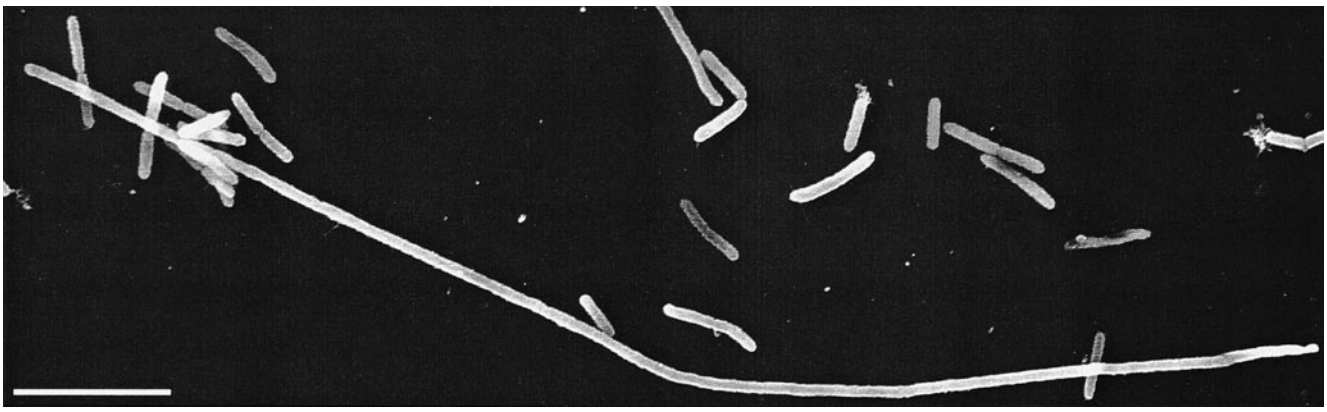

Fig. 2. Scanning electron micrograph of *Thermocrinis albus* (isolate HI 11/12) showing a 45-µm-long filament from the whitish streamer network formed at the overflow of the growth chamber. *Bar* 5 µm

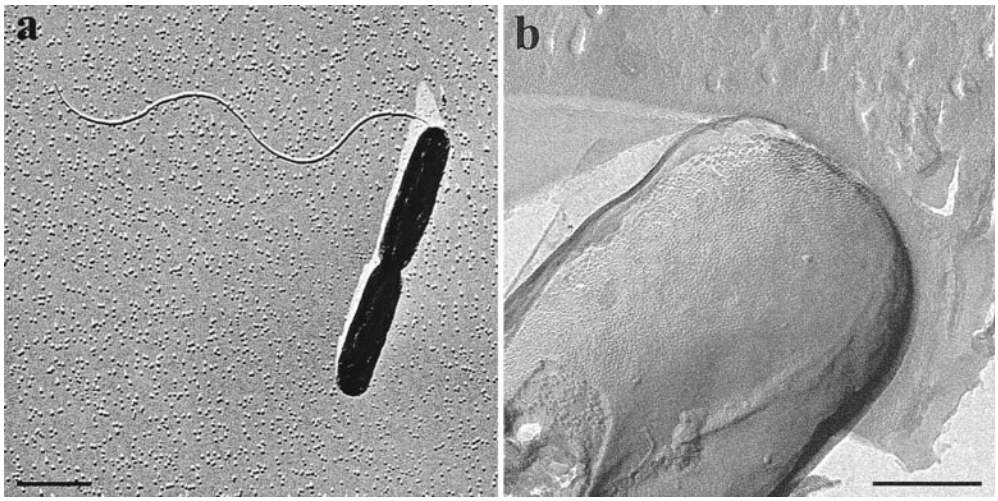

Fig. 3a,b. Transmission electron micrographs of *Thermocrinis albus* (isolate HI 11/12). **a** Flagellated cell in division, platinum shadowed. *Bar* 1 µm; **b** surface of a freeze-etched cell. *Bar* 0.2 µm

6 days, a white network of cells had formed, which consisted of long filaments with a length of 10–60 µm (Fig. 2). In addition, motile cells were present (also in the liquid phase of the glass chamber). The filamentous growth of isolate HI 11/12 and the whitish color of the cell network reflects the natural growth behavior of the organism in the hot streamlet of the Hveragerthi area in Iceland.

When grown in static culture, isolate HI 11/12 grew singly or in pairs. The average cell length was 1–3 µm, and the average cell width was 0.5–0.6 µm. Spores were not formed. In transmission electron micrographs, isolate HI 11/12 exhibited monopolar monotrichous flagellation (Fig. 3a). Freeze-etched cells (Fig. 3b) showed no evidence of the well-ordered two-dimensional crystalline arrays of S-layer proteins.

Metabolic properties

Like the known *Aquificales* strains, the new isolates grew chemolithoautotrophically under microaerophilic culture conditions with oxygen as the electron acceptor. Growth conditions of the new isolates are listed in Table 2 . More detailed growth studies were carried out with *A. pyrophilus*, *A. aeolicus*, *T. ruber*, isolate HI 11/12, and *H. thermophilus* TK-6.

Autotrophic growth

Isolate HI 11/12 grew optimally under microaerophilic conditions when hydrogen and sulfur were provided simultaneously as electron donors. Similar to *T. ruber*, *A. pyrophilus*, and *H. thermophilus* TK-6, isolate HI 11/12 could use hydrogen, thiosulfate, or sulfur as the sole electron donor for growth (Kawasumi et al. 1984; Bonjour and Aragno 1986; Huber et al. 1992, 1998). In the presence of sulfur, hydrogen sulfide was formed under low oxygen tension, as has been described for *T. ruber* and the *Aquifex* species (Huber et al. 1992, 1998; Huber and Stetter, Encyclopedia of Life Sciences: *Aquificales* http://www.els.net). In contrast to *A. pyrophilus*, the growth of *A. aeolicus* is obligately dependent on hydrogen (Huber and Stetter 2001).

The first representative of the *Aquificales* that was shown to grow anaerobically by nitrate reduction with N_2 as the final product (denitrification) was *A. pyrophilus* (Huber et al. 1992). More recently, it was shown that *H. thermophilus* TK-6 is also able to use nitrate as electron acceptor (Suzuki et al. 2001). This is also true for *Hydrogenobacter* sp. PA14

(TK-H medium + 0.1% KNO_3, wt/vol, 300 kPa H_2/CO_2; Kawasumi et al. 1980) isolated from Lake Tanganyika (Table 2 ; Tanganydro Group 1992; Tiercelin et al. 1993). As in the case of *T. ruber*, no growth on nitrate was observed for *A. aeolicus* or isolate HI 11/12.

Heterotrophic growth

T. ruber was the first organism observed to show chemo-organoheterotrophic growth on formate and formamide under microaerophilic culture conditions (Huber et al. 1998). In contrast, the following C_1 compounds were not metabolized by *T. ruber* (gas phase: $N_2 : O_2$, 97 : 3, vol/vol): formaldehyde (0.001%–0.1%, wt/vol), methanol (0.05%–0.5%, vol/vol), CO (1%–25%, vol/vol), or CH_4 (1%–20%, vol/vol). Like *T. ruber*, *H. thermophilus* TK-6 was also able to grow on formate and formamide (final concentration: 0.1%, wt/vol), but not on formaldehyde (0.001%–0.1%, wt/vol). The *Aquifex* species and isolate HI 11/12 were unable to metabolize formaldehyde (0.001%–0.1%, wt/vol), formate (0.1%, wt/vol), or formamide (0.1%, wt/vol) either microaerophilically with oxygen or anaerobically with nitrate as the electron acceptor. Furthermore, *H. thermophilus* TK-6 was unable to grow anaerobically with nitrate in the presence of formaldehyde, formate, or formamide (for concentrations, see above).

Physiological characteristics

Isolate HI 11/12 grew at temperatures between 55° and 89°C. No growth was obtained below 50°C or above 90°C. The maximum salt concentration was 0.7% NaCl. The isolate's close relatives G3L1B and UZ23L3A grew at temperatures between 44° and 88°C. No growth was obtained below 40° or above 90°C.

Biochemical properties

meso-Diaminopimelic acid was detected in several of the new strains. As in *Thermocrinis ruber* and the *Aquifex* strains, *meso*-diaminopimelic acid was detected in cell hydrolysates of isolate HI 11/12 (R_f 0.21) and in other related isolates, G3L1B (R_f 0.17) and UZ23L3A (R_f 0.18). Diaminopimelic acid was not present in hydrolysates of *Hydrogenobacter* sp. UZ27L2A, as has also been shown recently for other *Hydrogenobacter* species (Huber et al. 1998).

The fatty acids of isolate HI 11/12 were dominated by *n*-$C_{20:1}$ and *cy*-C_{21} compounds with subordinate $C_{18:0}$, $C_{18:1}$, *cy*-C_{19}, and $C_{20:0}$ (Jahnke et al. 2001). Furthermore, alkyl glycerol ethers, which were composed mainly of $C_{18:0}$ and $C_{20:1}$ monoethers, were present (Jahnke et al. 2001).

T. ruber forms pink colored cell masses either cultivated in batch culture or in a growth chamber under a permanent flow of medium (Huber et al. 1998). By using light–dark experiments (Huber et al. 1998), differential centrifugation, and energy dispersive X-ray analysis, it was demonstrated that the formation of pink pigment is independent of the light regime, the electron donor (hydrogen, sulfur, thiosulfate, or formate), or the oxygen concentration (0.05%–3% O_2, vol/vol, for static grown cultures or for cultures that have contact with air in the growth chamber).

DNA base composition

Total DNA of isolate HI 11/12 had a G + C content of 49.6 mol%, as calculated by direct analysis of the mononucleosides.

Discussion

Phylogenetic analyses showed that the *Aquificaceae* comprise four major sequence clusters represented by the genera *Aquifex*, *Thermocrinis*, *Hydrogenobacter*, and *Hydrogenobaculum* (Fig. 1). The marine *Aquifex* species are phylogenetically well separated from the low-salt-adapted members of *Thermocrinis* and *Hydrogenobacter*. However, *Hydrogenobaculum acidophilum* represents a separate branch within the *Aquificaceae* with no close relationship to the other members of the family (Fig. 1). Interestingly, isolate NOR3L3B (*Hydrogenobaculum* sp.) was isolated from an acidic (pH 3) hot spring of the Norris Geyser Basin of Yellowstone National Park (Wyoming, USA), suggesting that the acidophilic *Aquificales*, which group together in the phylogenetic analysis, are more abundant in high-temperature ecosystems. The environmental sequence cluster, containing sequences from hot springs in Japan, Iceland, the United States, and the deep sea (including *Hydrogenothermus marinus* and isolate EX-H1) forms a separate branch within the *Aquificales*, representing most likely a second family (Hugenholtz et al. 1998; Yamamoto et al. 1998; Reysenbach et al. 2000a, b, c; Skirnisdottir et al. 2000; Stöhr et al. 2001). We propose the name *Hydrogenothermaceae* for the new phylogenetic lineage.

By cultivation experiments, followed by phylogenetic analyses of the pure cultures obtained by the selected cell cultivation technique, novel representatives within all the major lineages of the *Aquificaceae* were obtained from various geothermal areas on earth (Fig. 1). Isolate HI 11/12, which was obtained from whitish streamers at Hveragerthi (Iceland), represents a novel branch within the *Aquificaceae* with a very close relationship to clone sequences identified in white and blue streamers from hot springs in Iceland (Skirnisdottir et al. 2000; Takacs et al. 2001). Further close relatives of HI 11/12 were found in Graendalur (Iceland) and in the Uzon Valley (Kamchatka). However, the 16S rRNA data of all *Aquificaceae* are not consistent with the distinct DNA–DNA similarity groups reflecting the geographical distribution of members of the *Aquificaceae* as proposed by Aragno (Aragno 1992).

Like *T. ruber*, isolate HI 11/12 grew in filamentous streamers when we created conditions resembling those present in the natural environment. These results demonstrate that filament formation by HI 11/12 is directly related to a permanent water current, as has already been shown for *T. ruber* (Huber et al. 1998). In contrast to *T. ruber*, how-

ever, the new isolate builds up a white network of cells. Comparative studies of pure cultures (Palleroni 1997) of physiologically different filament-forming organisms at temperatures up to 89°C gives us the opportunity to investigate filament formation under a permanent water current in more detail in the future. Thus far, *T. ruber* and isolate HI 11/12 represent filament-forming organisms with the highest growth temperatures known to date (Setchell 1903; Brock 1978; Huber et al. 1998). On the basis of morphological and phylogenetic properties, we propose the name *Thermocrinis albus*, or hot white hair, for isolate HI 11/12 as a second species within the genus *Thermocrinis*.

On the basis of the lipid analysis, *T. albus* shows a distribution of fatty acids and glycerol monoethers very similar to that shown recently for *T. ruber* and *H. thermophilus* TK-6 (Jahnke et al. 2001). No glycerol diethers, which are indicative of members of the genus *Aquifex*, are present (Jahnke et al. 2001). In contrast to the distribution of ether lipids within the *Aquificaceae*, the presence of *meso*-diaminopimelic acid seems to be restricted to members of the genera *Aquifex* and *Thermocrinis*. The detailed fatty acid analysis of different *Aquificaceae* representatives revealed that the *Aquificaceae* are a monophyletic lineage within the bacterial domain (consistent with the 16S rRNA data). These analyses suggested the unusual fatty acid *cy*-C_{21} as a group-specific biomarker (Jahnke et al. 2001).

The metabolic features within the *Aquificales* are not as clear cut as other properties within the order. All the new isolates and the other *Aquificales* members (except *H. subterraneus*; Takai et al. 2001) are capable of a chemolithoautotrophic growth under microaerophilic conditions with oxygen as the electron acceptor. As for *A. pyrophilus*, it was demonstrated that *Hydrogenobacter* sp. PA14 is also able to grow on nitrate under anaerobic conditions. Very recently, anaerobic nitrate reduction was also shown for *H. thermophilus* TK-6 (Suzuki et al. 2001). However, the *Thermocrinis* species are unable to reduce nitrate. Moreover, chemoorganoheterotrophic growth, which was first observed within the *Aquificales* for *T. ruber*, has now been demonstrated also for *H. thermophilus* TK-6, which is also able to grow on formate and formamide. This metabolic flexibility makes the organisms independent of inorganic growth substrates in nature and indicates a greater metabolic potential of the *Aquificales*.

To summarize, the isolation of novel *Aquificales* representatives from geographically well-separated geothermal and volcanic environments on earth increases our knowledge of the diversity and distribution of these organisms and strengthens the hypothesis that they play an important role in high-temperature ecosystems. Detailed studies of their metabolic and physiological potential provide an insight into the participation of *Aquificales* members in global biogeochemical cycles.

Description of *Thermocrinis albus*

Thermocrinis albus sp. nov. (al'bus. gr. *alphos*, white, referring to the cell color). Rod-shaped cells are usually between 1 and 3 μm long and 0.5 to 0.6 μm wide. Spores are not formed. Motile by means of a monopolar monotrichous flagellum. Cells occur singly, in pairs, and in aggregates consisting of up to several hundred individuals. In a permanent flow of medium, cells grow predominately as long filaments, forming visible whitish cell masses. No evidence of a regularly arrayed surface layer protein. Growth occurred at temperatures between 55° and 89°C and at salinities up to 0.7% NaCl. Aerobic. Chemolithoautotrophic. Molecular hydrogen, thiosulfate, and elemental sulfur serve as electron donors, and oxygen serves as an electron acceptor. *meso*-Diaminopimelic acid is present. Main fatty acids are $C_{18:0}$, $C_{18:1}$, *cy*-C_{19}, C_{20}, *n*-$C_{20:1}$, and *cy*-C_{21}. $C_{18:0}$ and $C_{20:1}$ alkyl glycerol monoethers are present. The G+C content is 49.6 mol%, as calculated by direct analysis of the mononucleosides. The type strain is *Thermocrinis albus* HI 11/12 (=DSM 14484, JCM 11386), which was isolated from grayish filaments collected in the Hveragerthi area, Iceland.

Description of *Hydrogenothermaceae*

Hydrogenothermaceae fam. nov. (Hy.dro.ge.no.ther.ma'ce.ae. Gr. N.L. masc. n. *Hydrogenothermus* type genus of the family; -aceae ending to denote the family; N.L. fem. pl. n. *Hydrogenothermaceae* the family of *Hydrogenothermus*) The *Hydrogenothermaceae* represent, next to the *Aquificaceae*, a separate phylogenetic branch within the *Aquificales*. Thermophilic motile rods var from 2 to 4 μm in length; Gram negative; spores not formed. Capable of chemolithotrophic microaerophilic growth using H_2, O_2, and CO_2. Optimal growth at 65°C. Type genus: *Hydrogenothermus* (Stöhr et al. 2001, p. 1860).

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References

Allen MB (1959) Studies with *Cyanidium caldarium*, an anomalously pigmented chlorophyte. Arch Mikrobiol 32:270–277

Aragno M (1992) Thermophilic, aerobic, hydrogen-oxidizing (Knallgas) bacteria. In: Balows A, Trüper HG, Dworkin M, Harder W, Schleifer KH (eds) The prokaryotes (2nd ed.). Springer, New York, pp 3917–3933

Barns SM, Fundyga RE, Jeffries MW, Pace NR (1994) Remarkable archaeal diversity detected in a Yellowstone National Park hot spring environment. Proc Natl Acad Sci U S A 91:1609–1613

Beck P, Huber R (1997) Detection of cell viability in cultures of hyperthermophiles. FEMS Microbiol Lett 147:11–14

Beffa T, Blanc M, Aragno M (1996) Obligately and facultatively autotrophic, sulfur- and hydrogen-oxidizing thermophilic bacteria isolated from hot composts. Arch Microbiol 165:34–40

Bonjour F, Aragno M (1986) Growth of thermophilic, obligatorily chemolithoautotrophic hydrogen-oxidizing bacteria related to

Hydrogenobacter with thiosulfate and elemental sulfur as electron and energy source. FEMS Microbiol Lett 35:11–15
Brock TD (1978) Thermophilic microorganisms and life at high temperatures. Springer, New York
Brock TD (1995) The road to Yellowstone and beyond. Annu Rev Microbiol 49:1–28
Brosius J, Dull TJ, Sleeter DD, Noller HF (1981) Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. J Mol Biol 148:107–127
Burggraf S, Olsen GJ, Stetter KO, Woese CR (1992) A phylogenetic analysis of *Aquifex pyrophilus*. Syst Appl Microbiol 15:352–356
Eder W, Ludwig W, Huber R (1999) Novel 16S rRNA gene sequences retrieved from highly saline brine sediments of Kebrit Deep, Red Sea. Arch Microbiol 172:213–218
Huber R, Stetter KO (2001) Genus *Aquifex*. In: Garrity G (ed) Bergey's manual of systematic bacteriology, vol 1, the archaea, cyanobacteria, phototrophs and deeply branching genera. Springer, New York, pp 360–362
Huber R, Woese CR, Langworthy TA, Kristjansson JK, Stetter KO (1990) *Fervidobacterium islandicum* sp. nov., a new extremely thermophilic eubacterium belonging to the "Thermotogales." Arch Microbiol 154:105–111
Huber R, Wilharm T, Huber D, Trincone A, Burggraf S, König H, Rachel R, Rockinger I, Fricke H, Stetter KO (1992) *Aquifex pyrophilus* gen. nov., sp. nov., represents a novel group of marine hyperthermophilic hydrogen-oxidizing bacteria. Syst Appl Microbiol 15:340–351
Huber R, Burggraf S, Mayer T, Barns SM, Rossnagel P, Stetter KO (1995) Isolation of a hyperthermophilic archaeum predicted by in situ RNA analysis. Nature 376:57–58
Huber R, Eder W, Heldwein S, Wanner G, Huber H, Rachel R, Stetter KO (1998) *Thermocrinis ruber* gen. nov., sp. nov., a pink-filament-forming hyperthermophilic bacterium isolated from Yellowstone National Park. Appl Environ Microbiol 64:3576–3583
Huber R, Huber H, Stetter KO (2000) Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties. FEMS Microbiol Rev 24:615–623
Huber R, Eder W, Stetter KO (2001) Genus *Thermocrinis*. In: Garrity G (ed) Bergey's manual of systematic bacteriology, vol 1, the archaea, cyanobacteria, phototrophs and deeply branching genera. Springer, New York, pp 364–366
Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1998) Novel division-level bacterial diversity in a Yellowstone hot spring. J Bacteriol 180:366–376
Ishii M, Igarashi Y, Kodama T (1987) Colony formation of *Hydrogenobacter thermophilus* on a plate solidified with Gelrite. Agric Biol Chem 51:3139–3141
Jahnke LL, Eder W, Huber R, Hope JM, Hinrichs K-U, Hayes JM, Des Marais DJ, Cady SL, Summons RE (2001) Composition of hydrothermal vent microbial communities as revealed by analyses of signature lipids, stable carbon isotopes and Aquificales cultures. Appl Environ Microbiol 67:5179–5189
Kawasumi T, Igarashi J, Kodama T, Minoda T (1980) Isolation of strictly thermophilic and obligately autotrophic hydrogen bacteria. Agric Biol Chem 44:1985–1986
Kawasumi T, Igarashi Y, Kodama T, Minoda Y (1984) *Hydrogenobacter thermophilus* gen. nov., sp. nov., an extremely thermophilic, aerobic, hydrogen-oxidizing bacterium. Int J Syst Bacteriol 34:5–10
Kristjansson KJ, Ingason A, Alfredsson GA (1985) Isolation of thermophilic obligately autotrophic hydrogen-oxidizing bacteria, similar to *Hydrogenobacter thermophilus*, from Icelandic hot springs. Arch Microbiol 140:321–325
Kryukov VR, Savel'eva ND, Pusheva MA (1983) *Calderobacterium hydrogenophilum* nov. gen., sp. nov., an extremely thermophilic hydrogen bacterium and its hydrogenase activity. Microbiology (Mikrobiologiya) 52:611–618
Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, Chichester, England, pp 115–175
Ludwig W (1995) Sequence databases (3.3.5). In: Akkermans ADL, Van Elsas JD, De Bruijn FJ (eds) Molecular microbial ecology manual. Kluwer Academic, Dordrecht, The Netherlands, pp 1–22
Ludwig W, Strunk O, Klugbauer S, Klugbauer N, Weizenegger M, Neumaier J, Bachleitner M, Schleifer K-H (1998) Bacterial phylogeny based on comparative sequence analysis. Electrophoresis 19:554–568
Maidak BL, Cole JR, Lilburn TG, Parker CTJ, Saxman PR, Stredwick JM, Garrity GM, Li B, Olsen GJ, Pramanik S, Schmidt TM, Tiedje JM (2000) The RDP (Ribosomal Database Project) continues. Nucleic Acids Res 28:173–174
Marler GD (1973) Inventory of thermal features of the Firehole River Geyser basins and other selected areas of Yellowstone National Park (PB-221289). U.S. Dept. of Commerce, National Technical Information Service, Springfield, VA, USA
Marteinsson VT, Kristjansson, JK, Kristmannsdottir H, Dahlkvist M, Saemundsson K, Hannigton M, Petursdottir SK, Geptner A, Stoffers P (2001) Discovery and description of giant submarine smectite cones on the seafloor in Eyjafjordur, northern Iceland, and a novel thermal habitat. Appl Environ Microbiol 67:827–833
Page RDM, Holmes EC (1998) Molecular evolution: a phylogenetic approach. Blackwell, Oxford, UK
Palleroni NJ (1997) Prokaryotic diversity and the importance of culturing. Antonie van Leeuwenhoek 72:3–19
Pitulle C, Yinqing Y, Marchiani M, Moore ERB, Siefert JL, Aragno M, Jurtshuk P, Fox GE (1994) Phylogenetic position of the genus *Hydrogenobacter*. Int J Syst Bacteriol 44:620–626
Reysenbach AL, Whickham GS, Pace NR (1994) Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. Appl Environ Microbiol 60:2113–2119
Reysenbach A-L, Banta AB, Boone DR, Cary SC, Luther GW (2000a) Microbial essentials at hydrothermal vents. Nature 404:835
Reysenbach A-L, Ehringer M, Hershberger K (2000b) 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, Longnecker K, Kirshtein J (2000c) Novel bacterial and archaeal lineages from an in situ growth chamber deployed at a Mid-Atlantic ridge hydrothermal vent. Appl Environ Microbiol 66:3798–3806
Rhuland LE, Work E, Denman RF, Hoare DS (1955) The behaviour of the isomers of α, ε-diaminopimelic acid on paper chromatograms. J Am Chem Soc 77:4844–4846
Setchell WA (1903) The upper temperature limits of life. Science 17:934–937
Shima S, Suzuki KI (1993) *Hydrogenobacter acidophilus* sp. nov., a thermoacidophilic, aerobic, hydrogen-oxidizing bacterium requiring elemental sulfur for growth. Int J Syst Bacteriol 43:703–708
Shima S, Yanagi M, Saiki H (1994) The phylogenetic position of *Hydrogenobacter acidophilus* based on 16S rRNA sequence analysis. FEMS Microbiol Lett 119:119–122
Skirnisdottir S, Hreggvidsson GO, Hjörleifsdottir S, Marteinsson VT, Petursdottir, SK, Holst O, Kristjansson JK (2000) Influence of sulfide and temperature on species composition and community structure of hot spring microbial mats, Appl Environ Microbiol 66: 2835–2841
Stöhr R, Waberski A, Völker H, Tindall BJ, 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
Suzuki M, Cui ZJ, Ishii M, Igarashi Y (2001) Nitrate respiratory metabolism in an obligately autotrophic hydrogen-oxidizing bacterium, *Hydrogenobacter thermophilus* TK-6. Arch Microbiol 175:75–78
Takacs CD, 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, Komatsu T, Horikoshi K (2001) *Hydrogenobacter subterraneus* sp. nov., an extremely thermophilic, heterotrophic bacterium unable to grow on hydrogen gas, from deep subsurface geothermal water. Int J Syst Evol Microbiol 51:1425–1435

Tanganydro Group (1992) Sublacustrine hydrothermal seeps in northern Lake Tanganyika, East African Rift: 1991 Tanganydro expedition. BCREDP 16:55–81

Tiercelin J-J, Pflumio C, Castrec M, Boulègue J, Gente P, Rolet J, Coussement C, Stetter KO, Huber R, Buku S, Mifundu W (1993) Hydrothermal vents in Lake Tanganyika, East African Rift system. Geology 21:499–502

Yamamoto H, Hiraishi A, Kato K, Chiura HX, Maki Y, Shimizu A (1998) Phylogenetic evidence for the existence of novel thermophilic bacteria in hot spring sulfur-turf microbial mats in Japan. Appl Environ Microbiol 64:1680–1687