2010

Thermocrinis minervae sp. nov., A Hydrogen and Sulfur-oxidizing, Thermophilic Member of the Aquificales from a Costa Rican Terrestrial Hot Spring

Sara L. Caldwell  
Portland State University

Yitai Liu  
Portland State University

Isabel Ferrera  
Institut de Ciències del Mar-CMIMA

Terry Beveridge  
University of Guelph

Anna-Louise Reysenbach  
Portland State University, reysenbacha@pdx.edu

Follow this and additional works at: https://pdxscholar.library.pdx.edu/bio_fac

Part of the Bacteriology Commons, and the Environmental Microbiology and Microbial Ecology Commons

Let us know how access to this document benefits you.

Citation Details

This Post-Print is brought to you for free and open access. It has been accepted for inclusion in Biology Faculty Publications and Presentations by an authorized administrator of PDXScholar. Please contact us if we can make this document more accessible: pdxscholar@pdx.edu.
Thermocrinis minervae sp. nov., a hydrogen and sulfur-oxidizing, thermophilic Aquificales from a Costa Rican terrestrial hot spring

Sara L. Caldwell¹, Yitai Liu¹, Isabel Ferrera², Terry Beveridge³, and Anna-Louise Reysenbach¹*

¹Department of Biology, Portland State University, P.O. Box 751, Portland, OR, 97207-0751, USA
²Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar-CMIMA, CSIC Passeig Marítim de la Barceloneta 37-49, E08003 Barcelona, Catalunya, Spain
³Department of Microbiology, College of Biological Science, University of Guelph, Guelph, Ontario, Canada, N1G 2W1

* Corresponding author (reysenbacha@pdx.edu; Tel. +1 (503) 725-3864; Fax. +1 (503) 725-3888)

Running title: Thermocrinis minervae sp. nov.

Graphs showing the effect of temperature and pH on the growth of Thermocrinis minervae CR11ᵀ are available as supplementary material in IJSEM online.

The GenBank accession number for the partial 16S rRNA gene sequence of strain CR11ᵀ is AM260555.
Summary

A thermophilic bacterium, designated strain CR11\textsuperscript{T}, was isolated from a filamentous sample collected from a terrestrial hot spring on the southwestern foothills of the Rincón Volcano, Costa Rica. The Gram-negative cells are approximately 2.4-3.9 µm long, 0.5-0.6 µm wide, and are motile rods with polar flagella. Strain CR11\textsuperscript{T} grows between 65 °C and 85 °C (optimum 75 °C, doubling time 4.5 h) and between pH 4.8 and 7.8 (optimum between 5.9 and 6.5). The isolate grows chemolithotrophically with S\textsuperscript{0}, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}, or H\textsubscript{2} as the electron donor, and with O\textsubscript{2} (up to 16 % v/v) as the sole electron acceptor. The isolate can grow on mannose, glucose, maltose, succinate, peptone, Casamino acids, starch, citrate, and yeast extract in the presence of oxygen (4 %) and S\textsuperscript{0}. Growth only occurs at NaCl concentrations below 0.4 % (v/w). The G+C content of strain CR11\textsuperscript{T} is 40.3 mol%. Phylogenetic analysis of the 16S rRNA gene places the strain as a close relative of Thermocrinis ruber (95.7 % sequence similarity). Based on phylogenetic and physiological characteristics, we propose the name Thermocrinis minervae for the new isolate with CR11\textsuperscript{T} as the type strain (=DSM 19557\textsuperscript{T}, =ATCC BAA-1533\textsuperscript{T}).
Introduction

The order Aquificales, represented by the Aquificaceae, Hydrogenothermaceae and Desulfurobacteriaceae, are thermophilic Bacteria that are widely distributed in hydrothermal systems and include microaerophilic chemolithotrophs and heterotrophs. Phylogenetic analysis of 16S rRNA gene sequences places the Aquificales as one of the deeply-branching lineages within the Bacteria (Burggraf et al., 1992; Pitulle et al., 1994; Di Giulio, 2003 a, b, c; Barion et al., 2007).

The Aquificaceae includes the genera Aquifex, Thermocrinis, Hydrogenobacter, Hydrogenivirga, and Hydrogenobaculum. Aquifex pyrophilus, originally isolated from a submarine hydrothermal vent system in Iceland (Huber et al., 1992), was the first described representative of this family, although numerous described Hydrogenobacter strains (Kryukov et al., 1983; Kawasumi et al., 1984; Kristjansson et al., 1985; Nishihara et al., 1990) also grouped within the Aquificaceae once their 16S rRNA sequences were determined (Burggraf et al., 1992; Pitulle et al., 1994).

Another clade within the Aquificaceae was reported from a culture-independent, molecular phylogenetic assessment of the diversity associated with the pink filamentous streamers from Octopus Spring, Yellowstone National Park (Reysenbach et al., 1994). Following this study, Huber et al. (1998) isolated the dominant Aquificales from this hot spring on a medium containing organic acids and named it Thermocrinis ruber. Subsequently, numerous new Thermocrinis isolates, including T. albus, were obtained from hot springs in Russia, Iceland, and Yellowstone National Park (Eder and Huber, 2002).
Cultivation studies and associated geochemical analyses (Huber et al., 1998; Eder and Huber, 2002; Blank et al., 2002; Hall et al., 2008; Connon et al., 2008) suggest that the metabolic activities of this group may contribute significantly to biogeochemical cycling in certain hydrothermal systems. Like the other members of the Aquificales, T. ruber and T. albus are dominant primary producers (Blank et al., 2002; Eder and Huber, 2002) in many ecosystems where photosynthesis is limited by high temperatures. Their ability to oxidize sulfur in laboratory cultures (Huber et al., 1998; Eder and Huber, 2002) suggests that these bacteria may also contribute to sulfur cycling in these environments. Additionally, 16S rRNA gene studies of a biofilm associated with As (III) oxidation in the Alvord Hot Spring, Oregon, indicated the presence of bacteria related to Thermocrinis and other Aquificales genera (Connon et al., 2008).

T. ruber and T. albus share similar physiological properties, including pH, temperature, and salinity optima (Huber et al., 1998; Eder and Huber, 2002). Both isolates are able to oxidize hydrogen, sulfur, and thiosulfate with oxygen as the sole electron acceptor, and have similar distributions of fatty acids and glycerol monoethers (Jahnke et al., 2001). However, T. ruber can also grow heterotrophically with formate and formamide, while T. albus appears to be a strict chemolithoautotroph (Huber et al., 1998; Eder and Huber, 2002). Based on 16S rRNA gene sequences, these isolates are somewhat distantly related (5.1% sequence difference; Eder and Huber, 2002).

Here we report the isolation and characterization of a new species of Thermocrinis. This strain is the first member of the Aquificales isolated from Costa Rica and is capable of using a relatively
large number of organic carbon sources, further expanding the geographic range and metabolic
diversity of this group.

Sample collection, enrichment, and isolation
Filamentous biomass samples were collected aseptically from a thermal spring (93 °C, pH 7.0)
on the southwestern foothills of the Rincón de la Vieja Volcano, Costa Rica. A sub-sample was
inoculated into 5 ml modified MSH medium (pH 6.2) under a gas phase of CO₂:H₂ (20:80;
Aguiar et al., 2004). Prior to inoculation, O₂ (4 %) was added to the medium. Enrichments were
incubated at 80 °C, without agitation, until the tubes became turbid and contained motile rods
under phase contrast microscopy. These cultures were immediately transferred into the same
media and purified by multiple dilution-to-extinction serial transfers. Purity of the isolate was
determined by 16S rRNA gene sequencing. The resulting pure culture was designated strain
CR11T and was characterized further. The isolate grew better with S⁰ than with H₂ as the
electron donor. Therefore, unless otherwise noted, all subsequent growth experiments used a
modified MSH medium (Aguiar et al., 2004) supplemented with approximately 0.06 % (w/v) S⁰
and 4 % (v/v) O₂.

Morphology
Cells were routinely monitored under phase-contrast microscopy using an Olympus BX60
microscope. Electron microscopic examination was performed as previously described
(Nakagawa et al., 2005). Thin sections were prepared by treating fixed cells with 2 % (w/v)
-osmium tetroxide and en block staining with 2 % uranyl acetate as described in Beveridge et al.
(1994). Cells were then dehydrated in ethanol and embedded in LR White. Sectioned cells were
mounted on carbon- and Formvar-coated 200-mesh grids and stained with uranyl acetate and
lead citrate. To create negative stains, grids were coated with a thin cell suspension, dried, and
stained with 2 % uranyl acetate.

CR11^T cells are motile, Gram-negative rods that vary in length from approximately 2.4 to 3.9
µm and from 0.5 to 0.6 µm in width (Fig. 1C). Cells did not form filaments during growth
although we did not try to stimulate filament formation as reported by Huber et al.1998. The
Gram-negative envelope has only an outer membrane as the surface component (Fig. 1A).
Approximately 5 % of the cells observed by transmission electron microscopy also have
cytoplasmic structures with unknown function (Fig. 1B). These structures have been reported
previously in other Aquificales (Götz et al., 2002; Aguiar et al., 2004; Flores et al., 2008).
Transmission electron micrographs of negatively stained cells show amphitrichous flagella (Fig.
1C).

**Growth characteristics**

Growth of the isolate was determined by direct cell counts using a Petroff-Hauser counting
chamber and a phase-contrast microscope (Olympus BX60). All experiments were performed in
triplicate at optimum temperature and pH unless otherwise noted.

The isolate grew between 65 °C and 85 °C with optimum growth occurring at about 75 °C
(doubling time 4.5 h; supplementary Fig. 1 in IJSEM Online). This growth range is below the
temperature measured in the spring during sample collection. It is well-estalished that that
growth under laboratory conditions may not directly reflect the growth conditions in the
environment or alternatively, a lower temperature variant was selected for in this study. The
effect of pH on growth was determined by adjusting the media to different pH values using 10
mM acetate / acetic acid buffer (pH 4-5), MES (pH 5-6.5), HEPES (pH 7), PIPES (pH 7-7.5),
and Tris (pH 7.5-8.0). CR11\textsuperscript{T} grew between pH 4.8 and 7.8 and optimally between 5.9 and 6.5
(supplementary Fig. 1B in IJSEM Online). No growth occurred below pH 4.8 or above 7.8. NaCl
requirements for growth of the isolate were determined from 0 to 1 % NaCl (w/v) in modified
MSH medium. The isolate grew in media containing 0-0.4 % NaCl.

Electron donors and acceptors were added to modified MSH medium without S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} (since it
may be used as an electron donor) and containing a reduced concentration of MgSO\textsubscript{4}·7H\textsubscript{2}O (4 g
l\textsuperscript{-1}). Electron couples were added aseptically after autoclaving and at concentrations reported in
Aguiar \textit{et al.} (2004). Media used for determining growth of CR11\textsuperscript{T} with H\textsubscript{2} as the electron donor
were prepared with H\textsubscript{2}:CO\textsubscript{2} (80:20) as the gas phase and with S\textsuperscript{o}, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-}, NO\textsubscript{3}\textsuperscript{-}, SO\textsubscript{3}\textsuperscript{2-}, SO\textsubscript{4}\textsuperscript{2-},
arsenate (as Na\textsubscript{2}HAsO\textsubscript{4}·7H\textsubscript{2}O), arsenite (as NaAsO\textsubscript{2}), selenate (as Na\textsubscript{2}SeO\textsubscript{4}), or selenite (as
Na\textsubscript{2}SeO\textsubscript{3}) as the electron acceptor. Growth with all other electron couples was determined using
media with a gas phase of N\textsubscript{2}:CO\textsubscript{2} (80:20). Electron donors and acceptors were added to this
media as follows: S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} / Fe\textsuperscript{3+} (as ferric citrate), Fe\textsuperscript{2+} (as FeCl\textsubscript{2}) / O\textsubscript{2}, Fe\textsuperscript{2+} (as FeCl\textsubscript{2}) / NO\textsubscript{3}\textsuperscript{-},
S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} / NO\textsubscript{3}\textsuperscript{-}, NH\textsubscript{4}\textsuperscript{+} / O\textsubscript{2}, NH\textsubscript{4}\textsuperscript{+} / NO\textsubscript{2}\textsuperscript{-}, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} / SO\textsubscript{4}\textsuperscript{2-}, SO\textsubscript{3}\textsuperscript{2-} / O\textsubscript{2}, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} / arsenate (as
Na\textsubscript{2}HAsO\textsubscript{4}·7H\textsubscript{2}O), arsenite (as NaAsO\textsubscript{2}) / O\textsubscript{2}, S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} / selenate (as Na\textsubscript{2}SeO\textsubscript{4}), S\textsuperscript{o} / selenite (as
Na\textsubscript{2}SeO\textsubscript{3}), S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} / selenite. CR11\textsuperscript{T} grew with H\textsubscript{2}, S\textsuperscript{o}, and S\textsubscript{2}O\textsubscript{3}\textsuperscript{2-} as electron donors and with O\textsubscript{2}
as the sole electron acceptor. The isolate grew with O\textsubscript{2} concentrations between 2 % and 16 %
(v/v). However, growth of the isolate was weak below 4 % and above 13 % (v/v) oxygen.
Heterotrophic growth of CR11T was determined by adding carbon sources at concentrations reported in Aguiar et al. (2004) to modified MSH medium containing no CO₂ or S₂O₃²⁻. Growth was monitored in the presence of O₂ as an electron acceptor and S⁰ as an electron donor. Cultures were also incubated in the absence of O₂ and S⁰ to test for fermentative growth. Cultures were transferred (5%) at least twice in the same substrate combinations to ensure that the cultures were not growing on the carried-over media. CR11T grew with 0.1 % mannose, glucose, maltose, succinate, Bacto peptone, Casamino acids, starch, citrate, and yeast extract as carbon sources with S⁰ as the electron donor and O₂ (4 % v/v) as the electron acceptor. No growth was detected under anaerobic conditions or in the absence of S⁰. Growth did not occur with sucrose, fructose, lactate, malate, oxalate, acetate, formaldehyde, propionate, sorbitol, methanol, tartaric acid, formamide, formate, or 2-propanol as the sole carbon sources.

DNA composition and phylogenetic analysis

DNA base composition (mol% G+C) was determined by thermal denaturation of genomic DNA (Marmur and Doty, 1962). DNA was extracted from a pure culture of CR11T (1 l) using the Qiagen Genomic-tip 100/G DNA extraction kit following the manufacturer’s protocol. The G+C content of CR11T is 40.3 mol%. This value is lower than the values reported for other Thermocrinis isolates (Table 1), but it is within the range of G+C content reported for other members of the Aquificaceae (the lowest reported value is for Hydrogenobaculum acidophilum, 35 mol%, Shima and Suzuki, 1993).

The 16S rRNA gene sequence was amplified by PCR and sequenced as described in Ferrera et al. (2007). The near full-length sequence of the 16S rRNA gene was assembled using
AutoAssembler (Applied Biosystems Inc.) and compared, using a BLAST search against the NCBI non-redundant database. The 16S rRNA gene sequence was manually aligned using the ARB program (Ludwig et al., 2004; http://www.mikro.biologie.tu-muenchen.de) based on the constraints of the secondary structure of the 16S rRNA molecule. The similarities in 16S rRNA gene sequences of CR11\textsuperscript{T} and the more closely-related members of the \textit{Aquificaceae} were calculated in ARB using 1469 homologous nucleotides within the \textit{Thermocrinis-} 
\textit{Hydrogenobacter} group. Phylogenetic trees were constructed in PAUP* (Swofford et al., 2003) using representative sequences of all members of the \textit{Aquificales} and including only unambiguously aligned nucleotides (1370 nt). Neighbor-joining (NJ; 1000 bootstrap replications) and maximum-likelihood (ML; 100 bootstrap replications) analyses were performed in ARB and PAUP* as previously described (Ferrera et al., 2007). Since the NJ and ML tree topologies are nearly identical, only the ML tree is shown in Fig. 2.

Comparison to related species

The 16S rRNA gene sequence analysis places strain CR11\textsuperscript{T} as a new species within the genus \textit{Thermocrinis} (70 % ML bootstrap value). CR11\textsuperscript{T} is most closely related to the environmental clone RIN3BA4 obtained from the same hot spring (Fig. 2). The closest described isolate to CR11\textsuperscript{T} is \textit{T. ruber} (95.7 % similar in 16S rRNA gene sequence). Strain CR11\textsuperscript{T} and RIN3BA4 form a separate lineage from \textit{T. ruber} and related strains. Together, these two lineages form a clade with \textit{Hydrogenobacter} (80 % ML bootstrap value), while the \textit{T. albus}-like group forms a separate monophyletic lineage (Fig. 2). Strain CR11\textsuperscript{T} is $> 95 \%$ similar in the 16S rRNA gene to most sequences of the \textit{T. ruber}-like clade. For example, CR11\textsuperscript{T} is 95.1 \% similar to the clone sequence EM17 (Reysenbach et al., 1994) and 95.2 % similar to \textit{Thermocrinis} sp. P2L2B (Eder
and Huber, 2002). The new isolate and clone RIN3BA4 are less than 95 % similar in the 16S rRNA gene (below the cutoff for genus level; Stackebrandt and Goebel, 1994) to all sequences within the *T. albus*-like clade (94.8 % similarity between CR11T and *T. albus*). The phylogenetic distance between these groups is similar to the previously reported distance of 5.1 %, based on maximum parsimony analysis, between *T. ruber* and *T. albus* (Eder and Huber, 2002).

The strains of *Thermocrinis* (including CR11T) are physiologically similar with respect to growth temperature ranges, low NaCl tolerances, and the electron donor/acceptor pairs used for chemolithotrophic growth (Table 1). However, they differ significantly in their ability to use organic carbon sources. Among these strains, CR11T appears to be metabolically more similar to *T. ruber* than to *T. albus*, in that CR11T is also capable of growing on organic carbon sources. However, CR11T grows on a greater diversity of organic carbon sources than *T. ruber* (Table 1). Furthermore, CR11T has a lower G+C (mol%) content than either *T. ruber* or *T. albus*.

Therefore, based on physiological and phylogenetic characteristics, we propose the new species, *Thermocrinis minervae*, with CR11T as the type strain (=DSM 19557T, =ATCC BAA-1533T).

**Description of *Thermocrinis minervae* sp. nov.**

*Thermocrinis minervae* (mi.ner'vae L. n. gen. fem. minervae, from Minerva, a Roman goddess, also known as Pallas Athena in Greek mythology, considered to be the virgin goddess of science, medicine, and wisdom).

Motile, Gram-negative rods with length approximately 2.4 to 3.9 µm and width 0.5-0.6 µm. Cells occur singly. Growth occurs between 65 and 85 °C (75 °C optimum), pH 4.8 to 7.8 (5.9-6.5
optimum), and NaCl concentrations from 0 to 0.4 % (w/v). Grows chemolithoautotrophically with H₂, S°, and S₂O₅²⁻ as electron donors and with only O₂ (up to 16% v/v) as the electron acceptor. Able to use yeast extract, mannose, glucose, maltose, succinate, peptone, Casamino acids, starch, citrate, and CO₂ as carbon sources. The G+C content of genomic DNA is 40.3 mol%. Isolated from a terrestrial hot spring on the southwestern foothills of the Rincón de la Vieja Volcano in Costa Rica. The GenBank accession number for the partial 16S rRNA gene sequence of strain CR11ᵀ is AM260555. The type strain is Thermocrinis minervae CR11ᵀ (=DSM 19557ᵀ, =ATCC BAA-1533ᵀ).

ACKNOWLEDGEMENTS

The authors thank Ana Sittenfeld for her assistance in sample collection. This work was supported by the grants NSF-PEET DEB-0328326 and NSF-MCB-0084224 awarded to ALR. S. Caldwell was supported by the OSU-PSU IGERT program, NSF-0114427. Electron microscopy was performed in the NSERC Guelph Regional Integrated Imaging Facility (GRIIF) which was partially funded by a NSERC Major Facility Access grant to TJB. The authors also wish to thank Dianne Moyles, University of Guelph, for her assistance with the electron microscopy.
References


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.


Table 1. Comparison of physiological properties and DNA base composition between CR11<sup>T</sup> and other described representatives of *Thermocrinis*. NR = not reported.

<table>
<thead>
<tr>
<th>Character</th>
<th>CR11&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thermocrinis ruber</em> OC 1/4&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thermocrinis albus</em> HI 11/12&lt;sup&gt;T&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin</td>
<td>Terrestrial hot spring, Costa Rica</td>
<td>Octopus Spring, Yellowstone National Park</td>
<td>Terrestrial hot spring, Iceland</td>
</tr>
<tr>
<td>Cell size (µm; length x width)</td>
<td>2.4-3.9 x 0.5-0.6</td>
<td>1-3 x 0.4</td>
<td>1-3 x 0.5-0.6</td>
</tr>
<tr>
<td>Temperature range (opt.) [°C]</td>
<td>65-85 (75)</td>
<td>44-89 (80)</td>
<td>55-89 (NR)</td>
</tr>
<tr>
<td>pH range (opt.)</td>
<td>4.8-7.8 (5.9-6.5)</td>
<td>Range NR (7 and 8.5)</td>
<td>Range NR (7)</td>
</tr>
<tr>
<td>NaCl range (opt.) [v/w]</td>
<td>0-0.4 % (NR)</td>
<td>0-0.4 % (NR)</td>
<td>&lt;0.7 % (NR)</td>
</tr>
<tr>
<td>Electron donors</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;, S°, S&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;, S°, S&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;, S°, S&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;</td>
</tr>
<tr>
<td>Electron acceptors</td>
<td>O&lt;sub&gt;2&lt;/sub&gt; (up to 16 % v/v)</td>
<td>O&lt;sub&gt;2&lt;/sub&gt; (up to 6 % v/v)</td>
<td>O&lt;sub&gt;2&lt;/sub&gt; (limit NR)</td>
</tr>
<tr>
<td>Organic carbon sources</td>
<td>Yeast, mannose, glucose, maltose, succinate, peptone, Casamino acids, starch, and citrate</td>
<td>Formate, formamide</td>
<td>None</td>
</tr>
<tr>
<td>G+C content (mol%)</td>
<td>40.3</td>
<td>47.2</td>
<td>49.6</td>
</tr>
</tbody>
</table>
Figure Legends

Fig. 1. Transmission electron micrographs of thin sections (A, B, and D) and a negatively stained cell (C) of CR11<sup>T</sup>. The arrow in B is pointing to the cytoplasmic structures with unknown function. The scale bar in A, B, and D is 0.5 µm; the scale bar in C is 2 µm.

Fig. 2. Maximum likelihood phylogenetic tree inferred from 16S rRNA gene sequences (1370 nt) showing the relative position of CR11<sup>T</sup> within the *Aquificaceae*. Bootstrap values correspond to 100 replicates. The tree topology was confirmed by the neighbor-joining algorithm.

Supplementary Fig. 1. Effects of temperature (A) and pH (B) on the growth of *Thermocrinis minervae* CR11<sup>T</sup>.
Figure 1
Figure 2

[Diagram showing phylogenetic relationships among various bacterial strains, including:
- Thermocrinis minervae CR11\(^T\) (AM2605555)
- Uncultured Thermocrinis clone RIN3BA4 (AM937264)
- Uncultured Thermocrinis EM17 (U05661)
- Uncultured Thermocrinis OP5 (AF018190)
- Thermocrinis sp. P2L2B (AJ320219)
- Thermocrinis ruber OC 1/4\(^T\) (AJ005640)
- Hydrogenobacter subterraneus HGP1\(^T\) (AB026268)
- Hydrogenobacter thermophilus TK-6\(^T\) (Z30189)
- Aquificales str. SRI-48 (AF255599)
- Uncultured Aquificales clone pIc1 (AF301907)
- Thermocrinis sp. UZ23L3A (AJ320220)
- Thermocrinis sp. G3L1B (AJ320221)
- Thermocrinis albus HI 11/12\(^T\) (AJ278895)
- Aquifex aeolicus VF5\(^T\) (AJ309733)
- Aquifex pyrophilus Kol5a\(^T\) (M83548)
- Hydrogenivirga caldivolida IBSK3\(^T\) (AB120294)\n]
Supplementary Figure 1

A

Specific growth rate (h⁻¹) vs. Temperature °C

B

Specific growth rate (h⁻¹) vs. pH