Archaeoglobus sulfaticallidus sp. nov., a thermophilic and facultatively lithoautotrophic sulfate-reducer isolated from black rust exposed to hot ridge flank crustal fluids

Bjørn O. Steinsbu,1,2 Ingunn H. Thorseth,1,2 Satoshi Nakagawa,3,† Fumio Inagaki,4 Mark A. Lever,5‡ Bert Engelen,6 Lise Øvreås1,7 and Rolf B. Pedersen1,2

1 Centre for Geobiology, University of Bergen, Allégaten 41, N-5007 Bergen, Norway
2 Department of Earth Science, University of Bergen, Allégaten 41, N-5007 Bergen, Norway
3 Subground Animalcule Retrieval (SUGAR) Program, Extremobiosphere Research Center (XBR), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), 2-15 Natsushima-cho, Yokosuka 237-0061, Japan
4 Kochi Institute for Core Sample Research, Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Monobe B200, Nankoku, Kochi 783-8502, Japan
5 Department of Marine Sciences, University of North Carolina at Chapel Hill, 12-7 Venable Hall CB #3300, Chapel Hill, North Carolina 27599, USA
6 Institut für Chemie und Biologie Des Meeres (ICBM), Carl von Ossietzky Universität Oldenburg, Carl von Ossietzky Straße 9-11, D-26111 Oldenburg, Germany
7 Department of Biology, University of Bergen, Jahnebakken 5, N-5007 Bergen, Norway

A novel thermophilic and lithoautotrophic sulfate-reducing archaeon was isolated from black rust formed on the steel surface of a borehole observatory (CORK 1026B) retrieved during IODP Expedition 301 on the eastern flank of Juan de Fuca Ridge, eastern Pacific Ocean. Cells of the strain were lobe-shaped or triangular. The optimum temperature, pH and NaCl concentration for growth were 75 °C, pH 7 and 2 % (w/v), respectively. The isolate was strictly anaerobic, growing lithoautotrophically on H2 and CO2 using sulfate, sulfite or thiosulfate as electron acceptors. Lactate and pyruvate could serve as alternative energy and carbon sources. The G+C content of the genomic DNA was 42 mol%. Phylogenetic analyses of the 16S rRNA gene indicated that the isolate was closely related to members of the family Archaeoglobaceae, with sequence similarities of 90.3–94.4 %. Physiological and molecular properties showed that the isolate represents a novel species of the genus Archaeoglobus. The name Archaeoglobus sulfaticallidus sp. nov. is proposed; the type strain is PM70-1T (=DSM 19444T =JCM 14716T).

Growth by dissimilatory sulfate reduction is widespread among the Bacteria and is conducted by psychrophilic and mesophilic, as well as thermophilic, species (Castro et al., 2000; Shen & Buick, 2004). Among members of the Archaea, the only sulfate-reducers described so far belong to the genera Archaeoglobus and Caldivirga. In contrast to the sulfate-reducing bacteria, they thrive in hyperthermophilic or extremely thermophilic conditions. The euryarchaeote Archaeoglobus fulgidus was the first sulfate-reducing archaeon to be described and it was isolated from geothermally heated sediments at Volcano, Italy (Stetter et al., 1987). Later, the hyperthermophilic and acidophilic sulfate-reducing crenarchaeote Caldivirga maquilingensis was isolated from a hot spring in the Philippines (Itoh et al., 1999). The genus Archaeoglobus presently consists of four species with validly published names: A. fulgidus (Stetter et al., 1987; Stetter, 1988), Archaeoglobus profundus (Burggraf et al., 1990), Archaeoglobus veneficus (Huber

Present address: Laboratory of Microbiology, Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041-8611, Japan.

Present address: Center for Geomicrobiology, Biology Institute, Aarhus University, 1540 Ny Munkegade, DK-8000 Aarhus C, Denmark.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PM70-1T is FJ810190.
et al., 1997) and Archaeoglobus infectus (Mori et al., 2008). An additional proposed member of the genus, ‘Archaeoglobus lithotrophicus’ (Stetter et al., 1993), has not yet been fully described and does not have a validly published name. All species of the genus Archaeoglobus characterized to date can use sulfur oxyanions as electron acceptors, producing H2S as an end product. They can all use sulfite or thiosulfate as electron acceptors and, except for A. veneficus and A. infectus, are ‘true’ sulfate reducers capable of using sulfate as an electron acceptor. Chemolithoautotrophic growth with sulfate has so far only been reported for ‘A. lithotrophicus’ (Harttzel & Reed, 2006). The other two genera belonging to the family, Ferroglobus and Geoglobus, are both autotrophic and unable to grow by reduction of sulfate or sulfite. Geoglobus anhangari (Kashefi et al., 2002a) and the recently described Geoglobus acetivorans (Slobodkina et al., 2009) can only use ferric iron as electron acceptor. Ferroglobus placidus was originally described as a nitrate- and thiosulfate-reducing chemolithoautotroph (Hafenbradl et al., 1996), but was later found to be capable of reducing ferric iron in the presence of aromatic compounds (Tor & Lovley, 2001) or acetate (Tor et al., 2001). All of the present type species of the family Archaeoglobaceae have been isolated from environments associated with oil reservoirs or hydrothermal vent systems. In this paper, a novel thermophilic and facultatively lithoautotrophic sulfate-reducer, strain PM70-1T, belonging to the genus Archaeoglobus is described.

Strain PM70-1T was enriched and isolated from black rust formations collected from the steel surface of a seafloor borehole observatory (CORK 1026B) retrieved during IODP Expedition 301 on the eastern flank of Juan de Fuca Ridge, eastern Pacific Ocean (Fisher et al., 2005; Nakagawa et al., 2006). The black rust was located just above the parts of the CORK designed to seal the top of a 295 m-deep borehole that was reaching through sediments and 48 m into basement. The rust had probably formed by the mixing of the cold surrounding seawater and hot (~64 °C) crustal fluids leaking through the seal (Nakagawa et al., 2006). Fresh samples of the black rust were crushed in a sterile mortar in an anaerobic glove box under an argon atmosphere. Slurries for cultivation were prepared by placing crushed sample material into 25 ml serum bottles (~15% of total volume) filled with 10 ml W20 medium (Lysnes et al., 2004). The bottles were sealed with butyl rubber stoppers and aluminium crimp caps and brought back to the laboratory at the University of Bergen without temperature control. The primary enrichment of PM70-1T was performed in a 25 ml bottle filled with 10 ml DSMZ 63-2 medium (Nakagawa et al., 2006) adjusted to pH 6.4 at room temperature. The bottle was inoculated with 1 ml of the black rust slurry and incubated at 70 °C without shaking. After 2 weeks of incubation, a black precipitate formed, indicating production of sulfide in the enrichment culture. Examination of the culture by light microscopy revealed irregular lobe-shaped and triangular cells, appearing singly or in pairs. The culture could be repeatedly transferred to fresh medium, but an observed lag phase of about 1 week followed by rapid growth indicated that the initial growth conditions were suboptimal. Strain PM70-1T was therefore isolated and routinely cultivated in HYL medium, which had the following composition (1 l−1): HEPES (Sigma, H3375), 9.52 g; NaCl, 20.0 g; Na2SO4, 4.0 g; MgCl2.6H2O, 4.3 g; NH4Cl, 0.25 g; KCl, 0.34 g; CaCl2.2H2O, 0.14 g; K2HPO4.3H2O, 0.14 g; yeast extract (Merck), 1.0 g; sodium lactate [50% (w/v) solution, Merck], 4.5 ml; resazurin, 0.001 g; Fe(NH4)2(SO4)2, 6H2O, 0.002 g and 1 ml trace element solution. The trace element solution, modified from Hartzell & Reed (2006), contained (1 l−1): Na2EDTA.2H2O, 29 g (pH 8); MnSO4.H2O, 3.26 g; CoCl2.6H2O, 1.8 g; ZnSO4.7H2O, 1.0 g; NiSO4.6H2O, 0.11 g; CuSO4.5H2O, 0.1 g; H3BO3, 0.1 g; KAl(SO4)2.12H2O, 0.1 g; Na2MoO4.2H2O, 0.1 g; Na2WO4.2H2O, 0.1 g and Na2SeO3, 0.05 g. The pH was adjusted to 7.2 at room temperature. After autoclaving in a dispenser (Widdel & Bak, 1992), the medium was cooled down under N2 gas and then 10 ml vitamin solution (Balch et al., 1979) and 4 ml 0.5 M Na2S, 9H2O were added from sterilized anaerobic stocks. The pH was readjusted to 7.2 and the medium was dispensed into 50 or 100 ml serum bottles or 27 ml Balch tubes (Bellco) under a gas phase of N2 (100 kPa). The bottles and tubes were then sealed with butyl rubber stoppers and aluminium crimp caps. A pure culture was obtained by serial dilution of the enrichment culture in HYL medium solidified with 0.3% Gelrite gellan gum (Sigma). Three colonies were picked; one from the tube with the highest dilution and two from the preceding tube in the series. Colonies were purified by repeating the dilution procedure twice. The purity of the strains was checked by phase-contrast microscopy and by PCR and sequencing of the 16S rRNA gene with several different primers. The three strains had identical 16S rRNA gene sequences and therefore only the type strain, designated PM70-1T, isolated from the tube with the highest dilution was characterized further.

The strain was strictly anaerobic and the presence of elemental sulfur inhibited growth. It was very sensitive to pH changes and had a narrow pH range for growth. Furthermore, the presence of 0.05–0.10% yeast extract was necessary for stable growth on organic substrates; otherwise a lag phase lasting for several days was observed. Yeast extract was not needed when the strain was grown on H2/CO2 (80:20, 200 kPa). Phase-contrast microscopy revealed that cells of strain PM70-1T were very flat, non-motile lobes or triangles. These morphologies were also evident when ultrathin sections of the cells were observed by transmission electron microscopy (Fig. 1). Short chains of up to five cells could sometimes be seen during exponential growth and a stalk-like structure was often observed between dividing cells. Cells showed blue-green fluorescence when exposed to UV under a fluorescence microscope, which indicated that they possessed a coenzyme F420-like compound.

The 16S rRNA gene of strain PM70-1T was amplified by whole-cell PCR with primers A20yrF [5′-TTCGGTGTGA-
post-fixed in 1 % (w/v) OsO₄, dehydrated in graded ethanol series from the same borehole (Cowen accession no. AY181046), which was retrieved from fluids the uncultured environmental clone 1026B270 (GenBank 1997) showed that the strain was most closely related to strain PM70-1T. Cells were fixed in 2.5 % (w/v) glutaraldehyde, et al. searches against sequences in GenBank (Altschul et al., 1997) to construct a distance tree using the Jukes–Cantor distance matrix (Jukes & Cantor, 1969) and the neighbour-joining algorithm (Saitou &Nei, 1987). Confidence in the tree topologies was established by bootstrap calculations (Felsenstein, 1985). All of the three different methods placed strain PM70-1(T and clone pMC2A228 on a common branch that was clearly separated from the other type species of the family Archaeoglobaceae. The maximum-likelihood tree and the Bayesian tree produced identical tree topologies, with A. infectus located on the branch closest to strain PM70-1(T (Fig. 2). The only change observed in the neighbour-joining tree (not shown) as compared with the presented tree was that A. veneficus and A. infectus grouped together on a branch located between the branch of ‘A. lithotrophicus’ and the branch containing strain PM70-1(T and clone pMC2A228. 16S rRNA gene sequence identities between strain PM70-1(T and other type strains in the family Archaeoglobaceae were calculated by global pairwise alignments in MatGAT 2.02 (Campanella et al., 2003). Because some of the sequences were slightly shorter than others, only the parts of the sequences corresponding to nucleotide positions 31–1435 of the deposited sequence (FJ810190) of strain PM70-1(T were considered. Sequence similarities between strain PM70-1(T and the type strains of A. veneficus, A. infectus, A. fulgidus, G. ahangari, G. acetivorans and F. placidus were 94.4, 93.8, 93.5, 90.3, 93.7, 92.8 and 90.8 %, respectively.

Phylogenetic analyses indicated that the taxonomic position of A. profundus should be reconsidered. The tree presented in Fig. 2 clearly demonstrates that A. profundus is more closely related to F. placidus than to the other species of the genus Archaeoglobus. The analyses performed with MatGAT showed that the 16S rRNA gene sequence similarity between the type strains of A. profundus and F. placidus was 96.3 %, a value that in many cases would be considered rather high for strains representing two different genera. In contrast, similarities between the type strains of A. profundus and the other type strains in the family Archaeoglobaceae were as low as 90.5–93.5 %, values that would normally indicate that a species represents a unique genus (Yarza et al., 2008). A reclassification of A. profundus is problematic as no really close relatives besides F. placidus have been described so far. Placing the two species in the same genus does not sound like a good solution as they possess very different physiological properties (Table 1). The physiological properties of A. profundus clearly indicate that the species does not merit.

Fig. 1. Transmission electron micrograph of an ultrathin section of strain PM70-1(T. Cells were fixed in 2.5 % (w/v) glutaraldehyde, post-fixed in 1 % (w/v) OsO₄, dehydrated in graded ethanol series and embedded in LR White epoxy resin. Ultrathin sections were cut in an ultramicrotome (Reichert-Jung), loaded onto FORMVAR-coated copper grids, and stained with 1 % (w/v) uranyl acetate and Reynolds lead citrate (Reynolds, 1963). Samples were examined using a JEM-1011 TEM operating at 80 kV accelerating voltage. Images were acquired with a Morada digital camera (Soft Imaging System). Bar, 1 μm.

TCCYGCCRG-3‘ (Massana et al., 1997) and Hr [5’-AAGGAGGTGATCCAGCCGCA-3’ (Edwards et al., 1989)], and sequenced and assembled by standard methods. BLAST searches against sequences in GenBank (Altschul et al., 1997) showed that the strain was most closely related to the uncultured environmental clone 1026B270 (GenBank accession no. AY181046), which was retrieved from fluids from the same borehole (Cowen et al., 2003). This clone sequence was, however, excluded from further phylogenetic analyses because of its short length (837 nt). Alignments and phylogenetic trees with sequences covering the almost complete 16S rRNA gene region of all type species in the family Archaeoglobaceae and representatives of other groups of the Archaea were made by use of the SINA webaligner available through the SILVA web page (http://www.arb-silva.de) (Pruesse et al., 2007). The alignment was exported to ARB 5.0 (Ludwig et al., 2004), where it was corrected manually. Phylogenetic analyses were performed by use of the ‘Phylogeny.fr’ web server (Dereeper et al., 2008) as follows. The alignment was further refined with Gblocks 0.91b (Castrasana, 2000) and the function ‘allow smaller final blocks’. A maximum-likelihood tree was constructed with PhyML 3.0 aLRT (Guindon & Gascuel, 2003) and by use of the ‘GTR’ substitution model assuming an estimated proportion of invariant sites and four gamma-distributed rate categories to account for rate heterogeneity across sites. A Bayesian inferred tree was made with MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) with the number of substitution types fixed to 6. The substitution model ‘4by4’ was used and rate variation across sites was fixed to ‘invariable + gamma’. Four ‘Markov Chain Monte Carlo’ chains were run for 10 000 generations. Sampling was performed every 10 generations and the first 250 sampled trees were discarded. Finally, a 50 % majority rule consensus tree was constructed. The refined alignment was also exported to MEGA4 (Tamura et al., 2007) to construct a distance tree using the Jukes–Cantor distance matrix (Jukes & Cantor, 1969) and the neighbour-joining algorithm (Saitou & Nei, 1987). Confidence in the tree topologies was established by bootstrap calculations (Felsenstein, 1985). All of the three different methods placed strain PM70-1(T and clone pMC2A228 on a common branch that was clearly separated from the other type species of the family Archaeoglobaceae. The maximum-likelihood tree and the Bayesian tree produced identical tree topologies, with A. infectus located on the branch closest to strain PM70-1(T (Fig. 2). The only change observed in the neighbour-joining tree (not shown) as compared with the presented tree was that A. veneficus and A. infectus grouped together on a branch located between the branch of ‘A. lithotrophicus’ and the branch containing strain PM70-1(T and clone pMC2A228. 16S rRNA gene sequence identities between strain PM70-1(T and other type strains in the family Archaeoglobaceae were calculated by global pairwise alignments in MatGAT 2.02 (Campanella et al., 2003). Because some of the sequences were slightly shorter than others, only the parts of the sequences corresponding to nucleotide positions 31–1435 of the deposited sequence (FJ810190) of strain PM70-1(T were considered. Sequence similarities between strain PM70-1(T and the type strains of A. veneficus, A. infectus, A. fulgidus, G. ahangari, G. acetivorans and F. placidus were 94.4, 93.8, 93.5, 90.3, 93.7, 92.8 and 90.8 %, respectively.

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classification as a member of the genus *Archaeoglobus*. We therefore think that emendation of the description of *A. profundus* should be postponed until more species have been described. Moreover, the genome sequences of the type strains of *A. profundus* and *F. placidus* will soon be available and will undoubtedly provide important information sustaining a more reliable and thorough reclassification.

Growth of strain PM70-1 T was determined by measuring changes in turbidity at 600 nm by placing culture tubes directly into a Spectronic 21 spectrophotometer (Milton Roy) or by cell counting in a Thoma chamber (0.02 μm depth). Sulfide produced in the culture tubes was detected as described by Cord-Ruwisch (1985) with medium from a tube without inoculum serving as a reference. Growth experiments were performed in Balch tubes (Bellco) filled with 10 ml medium. Growth on H₂ and CO₂ was tested using 100 ml bottles filled with 25 ml medium.

The influence of temperature on growth of strain PM70-1 T was determined in the range 50–85 °C in HYL medium. The pH range for growth was investigated at 75 °C using HYL medium buffered with 20 mM PIPES (Sigma) in the pH range 6.1–7.1 and with 20 mM HEPES (Sigma) in the pH range 7.0–7.8. The pH was adjusted at room temperature after autoclaving the medium. In order to determine the salt requirement, the strain was grown in HYL medium prepared with different amounts of NaCl and incubated at 75 °C and pH 7.0. Salt experiments were performed in duplicate, whereas pH experiments were performed in triplicate. Strain PM70-1 T grew at 60–80 °C (optimum growth at 75 °C), at pH 6.3–7.6 (optimum around pH 7.0) and in 0.5–3.5 % (w/v) NaCl (optimum around 2.0 %). No growth was detected at 55 or 82 °C, pH 6.1 or 7.8, or in the presence of 0 or 4.0 % NaCl. The strain was very sensitive to pH changes; at pH values between 6.3 and 6.6, a lag phase lasting for several days was observed, but after that rapid growth occurred. A slow increase in pH was observed during the lag phase indicating that the strain was probably able to produce sufficient amounts of sulfide to slowly raise pH towards the optimum value for growth.

To determine the ability of strain PM70-1 T to use alternative substrates and electron acceptors, both HEPES (20 mM) and NaHCO₃ (12 mM) buffered variants of the HYL medium were used, as HEPES is known for catalysing the reduction of ferric iron (Kashefi et al., 2002b). Besides the buffer change, the HYL medium was modified as follows: lactate, sulfate and yeast extract were omitted. When ferric iron species were tested as electron acceptors, Na₂S was replaced with 0.2 mM FeCl₂, which was added to each tube just before inoculation. Electron donors and acceptors were added to the modified medium in different combinations from anaerobic and separately sterilized stock solutions. Poorly crystalline FeOOH was prepared as described by Lovley (2006) and separately sterilized by autoclaving at 121 °C. Elemental sulfur (~0.2 g) was prepared in pre-sterilized Balch tubes which were flushed with N₂ and sealed with butyl rubber stoppers. Sterile distilled water (0.1 ml) was added with a syringe just before the tubes were heated in a water bath at 95 °C for 3 h. The heating step was repeated on two successive days. All tests were performed both with and without 0.1 % (w/v) yeast extract and were initially conducted without repetition. In cases where no growth was detected, the experiment was repeated in duplicate with fresh inoculum and medium. The strain was regarded as utilizing the added electron acceptors and donors only if growth was sustained after three subsequent transfers into fresh medium.
Table 1. Comparison of physiological characteristics of strain PM70-1T with those of other type strains of the family Archaeoglobaceae

Strains: 1, PM70-1T; 2, *A. infectus* Arc51T; 3, *A. veneficus* SNP6T; 4, *A. fulgidus* VC-16T; 5, *A. profundus* AV18T; 6, *G. ahangari* 234T; 7, *G. acetivorans* SBH6T; 8, *F. placidus* AEDII12DOT. Data for reference strains were taken from Mori et al. (2008), Huber et al. (1997), Stetter et al. (1987), Stetter (1988), Burggraf et al. (1990), Kashefi et al. (2002a), Slobodkina et al. (2009), Hafenbradl et al. (1996), Tor & Lovley (2001) and Tor et al. (2001). +, Positive; (+), weakly positive; −, negative; ND, not determined.

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<th>Characteristic</th>
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<td>Isolation source</td>
<td>Black rust formations on a deep-sea borehole observatory, Juan de Fuca Ridge</td>
<td>Deep-sea hydrothermal system, Suiyo Seamount, Izu-Bonin Arc, western Pacific Ocean</td>
<td>Deep-sea black smoker wall, ‘Snake Pit’ site, Mid-Atlantic Ridge</td>
<td>Hot sediment from shallow water hydrothermal system, Volcano, Italy</td>
<td>Deep-sea hydrothermal system, Guaymas, Mexico</td>
<td>Deep-sea hydrothermal system, Guaymas, Mexico</td>
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<td>H₂CO₂ + Fe³⁺</td>
<td>H₂CO₂ + Fe³⁺</td>
<td>H₂CO₂ + Fe³⁺</td>
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<td>+</td>
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</tr>
<tr>
<td>Lactate</td>
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<td>−</td>
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<td>−</td>
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<td>Yeast extract</td>
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<td>−</td>
<td>+</td>
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<td>−</td>
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Archaeoglobus sulfaticallidus sp. nov., a sulfate-reducer
PM70-1T could use sulfate (28 mM), sulfite (4 mM) and thiosulfate (20 mM) as electron acceptors and H\textsubscript{2}/CO\textsubscript{2} (80:20, v/v; 200 kPa), lactate (20 mM) and pyruvate (20 mM) as electron donors. Yeast extract stimulated growth, but could not be used as a substrate. No growth of \textit{A. fulgidus} VC-16\textsuperscript{T} was observed with H\textsubscript{2}/CO\textsubscript{2} and sulfate in the same medium, in the presence or absence of 0.03% yeast extract. On the other hand, strain VC-16\textsuperscript{T} grew very well on H\textsubscript{2}/CO\textsubscript{2} and thiosulfate. The following electron acceptors could not be utilized by strain PM70-1T: nitrate (10 mM), S\textsuperscript{0} (2%, w/v), iron(III) citrate (20 mM) and poorly crystalline FeOOH (100 mM). The following electron donors could not be used: formate (20 mM), acetate (20 mM), propionate (20 mM), butyrate (20 mM), citrate (20 mM), succinate (20 mM), fumarate (20 mM), malate (20 mM), methanol (20 mM), ethanol (20 mM), Casamino acids (0.1%), yeast extract (0.1 and 0.5%), D-glucose (0.2%) and starch (0.2%).

The DNA G+C content of strain PM70-1T was determined by the thermal denaturation (\textit{T\textsubscript{m}}) method (Mandel \textit{et al.}, 1970) on a Cary 4E spectrophotometer (Varian). Genomic DNA was isolated from frozen cell pellets according to Marmur (1963). \textit{Escherichia coli} DNA (Sigma D-2001) was used as a reference for the measurement. The DNA G+C content of strain PM70-1T was 42 mol%.

The differential characteristics of strain PM70-1T and the other type strains belonging to the family \textit{Archaeoglobaceae} are summarized in Table 1. Unlike \textit{G. ahangari} and \textit{G. acetivorans}, strain PM70-1T was unable to grow by iron reduction and, unlike \textit{F. placidus}, it could not grow by nitrate reduction. All family members, except \textit{A. profundus} and \textit{A. infectus}, could grow chemolithoautotrophically (Table 1). Strain PM70-1T shared this trait, but differed from the others by being able to couple it to sulfate reduction. Chemolithoautotrophic growth on H\textsubscript{2}/CO\textsubscript{2} and sulfate has so far only been described for \textit{A. lithotrophicus}, which, in contrast to strain PM70-1T, is not capable of heterotrophic growth. The salt and pH optima for growth of strain PM70-1T were similar to those of previously described species of the order \textit{Archaeoglobales}, with the exception of \textit{A. infectus}, which has a higher salt optimum than the other species. Like \textit{A. infectus}, strain PM70-1T was not able to grow at temperatures above 80°C, which separated these two species from the previously described hyperthermophilic members of the family. Moreover, the measured temperature, pH and salt ranges for these two species were generally narrower than those of the other species (Table 1). The optimal growth temperature for strain PM70-1T was midway between the optimum growth temperatures for \textit{A. infectus} and the other species of the genus \textit{Archaeoglobus}. Like \textit{A. veneficus} and \textit{A. fulgidus}, strain PM70-1T could grow by using organic acids as electron donors, but the substrate spectrum for strain PM70-1T was slightly different (Table 1). \textit{A. fulgidus} differed from the other two strains by its use of methanol, and from strain PM70-1T by its use of ethanol. Both \textit{A. fulgidus} and \textit{A. veneficus} were able to use formate and yeast.

<table>
<thead>
<tr>
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<th>Ethanol</th>
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<th>Fumarate</th>
</tr>
</thead>
<tbody>
<tr>
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<td>+</td>
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<tr>
<td>7</td>
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Table 1 cont.:

- Determined by HPLC.
- Only \textit{Fe}3+ used as electron acceptor.
- Only \textit{SO}2\textsuperscript{3-} used as electron acceptor.
- \textsuperscript{+}Determined by \textit{T\textsubscript{m}} method.
- \textsuperscript{+}Only \textit{Fe}2+ used as electron acceptor.
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extract as electron donors, in contrast to strain PM70-1<sup>T</sup>. Lactate could be used by strain PM70-1<sup>T</sup> and <i>A. fulgidus</i>, but not by <i>A. veneficus</i>. Moreover, DNA G+C contents, when determined by the <i>Tm</i> method, were 3 and 4 mol% lower for strain PM70-1<sup>T</sup> than for <i>A. veneficus</i> and <i>A. fulgidus</i>, respectively. The phylogenetic analyses unambiguously placed strain PM70-1<sup>T</sup> within the family <i>Archaeoglobaceae</i> (Fig. 2), and the phylogenetic distance to previously described species clearly indicated that the strain represents a novel species (Stackebrandt & Goebel, 1994; Stackebrandt & Ebers, 2006). Based on the data presented above, it is suggested that strain PM70-1<sup>T</sup> (=DSM 19444<sup>T</sup>=JCM 14716<sup>T</sup>) should be classified as a novel species for which the name <i>Archaeoglobus sulfaticallidus</i> sp. nov. is proposed.

**Description of <i>Archaeoglobus sulfaticallidus</i> sp. nov.**

<i>Archaeoglobus sulfaticallidus</i> (sul.fati.call’id.us. N.L. masc. n. <i>sulfat-</i> -atis sulfate; L. adj. callidus skilful; N.L. masc. adj. <i>sulfaticallidus</i> skilful with sulfate, referring to the strain’s capability of both chemolithoautotrophic and chemooorganotrophic growth on sulfate).

Cells are non-motile irregular lobes or triangles (0.4–2.2 μm in diameter), occurring singly or in pairs, but sometimes in short chains of up to five cells. Shows blue-green fluorescence under UV light. Strictly anaerobic; grows by reduction of sulfate, sulfite or thiosulfate in the presence of lactate, pyruvate or H<sub>2</sub>/CO<sub>2</sub>. Growth is by reduction of sulfate, sulfite or thiosulfate in the presence of lactate, pyruvate or H<sub>2</sub>/CO<sub>2</sub>. Growth is by reduction of sulfate, sulfite or thiosulfate in the presence of lactate, pyruvate or H<sub>2</sub>/CO<sub>2</sub>.

The type strain is PM70-1<sup>T</sup> (=DSM 19444<sup>T</sup>=JCM 14716<sup>T</sup>), isolated from black rust formed on the steel surface of a seafloor borehole observatory (ODP CORK 1026B, eastern flank of Juan de Fuca Ridge) emitting hot (~64 °C) crustal fluids (water depth 2658 m). The DNA G+C content of the type strain is 42 mol% (<i>Tm</i> method).

**Acknowledgements**

This research used samples provided by the Integrated Ocean Drilling Program (IODP). We would like to thank the crew of R/V JOIDES RESOLUTION, the chief scientists and the shipboard scientific party of IODP Leg 301 for valuable support during the cruise. Many thanks go to Frida-Lise Daae, who performed the DNA G+C analysis, and to Mikal Heldal and Irene Heggstad for the preparation of electron micrographs. This work was partly supported by a grant to B.O.S. from IODP Norway, and by the Norwegian Research Council through the ‘BioDeep’ project (grant no.160932).

**References**


