Acidianus sulfidivorans sp. nov., an extremely acidophilic, thermophilic archaeon isolated from a solfatara on Lihir Island, Papua New Guinea, and emendation of the genus description

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A novel, extremely thermoacidophilic, obligately chemolithotrophic archaeon (strain JP7^T) was isolated from a solfatara on Lihir Island, Papua New Guinea. Cells of this organism were non-motile, Gram-negative staining, irregular-shaped cocci, 0.5–1.5 µm in size, that grew aerobically by oxidation of sulfur, Fe²⁺ or mineral sulfides. Cells grew anaerobically using Fe³⁺ as a terminal electron acceptor and H₂S as an electron donor but did not oxidize hydrogen with elemental sulfur as electron acceptor. Strain JP7^T grew optimally at 74 °C (temperature range 45–83 °C) and pH 0.8–1.4 (pH range 0.35–3.0). On the basis of 16S rRNA gene sequence similarity, strain JP7^T was shown to belong to the *Sulfolobaceae*, being most closely related to the type strains of *Acidianus ambivalens* (93.7 %) and *Acidianus infernus* (93.6 %). Cell-membrane lipid structure, DNA base composition and 16S rRNA gene sequence similarity data support the placement of this strain in the genus *Acidianus*. Differences in aerobic and anaerobic metabolism, temperature and pH range for growth, and 16S rRNA gene sequence differentiate strain JP7^T from recognized species of the genus *Acidianus*, and an emendation of the description of the genus is proposed. Strain JP7^T is considered to represent a novel species of the genus *Acidianus*, for which the name *Acidianus sulfidivorans* sp. nov. is proposed. The type strain is JP7^T (=DSM 18786^T=JCM 13667^T).

The genus *Acidianus*, which was first described by Segerer *et al.* (1986), is a member of the order *Sulfolobales* (Stetter, 1989), and comprises three formally recognized species of facultatively aerobic thermoacidophiles isolated from geothermal or hydrothermal systems. The phylogenetically similar species *Acidianus infernus* and *Acidianus ambivalens* and the more distantly related and less thermophilic species *Acidianus brierleyi* (Fuchs *et al.*, 1996), originally named *Sulfolobus brierleyi* (Brierley & Brierley, 1973; Zillig *et al.*, 1980), grow as facultative aerobes with the ability to oxidize or reduce elemental sulfur depending on the available oxygen supply and are capable of autotrophic growth. Two recently described species, '*Acidianus tengchongensis*' and '*Acidianus manzaensis*', have extended the genotypic and phenotypic diversity within the genus (He *et al.*, 2004; Yoshida *et al.*, 2006).

Abbreviation: GDGTs, glycerol dialkyl glycerol tetraethers.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JP7^T is AY907891.

Moist, sulfur-rich soil was collected from the hot (>80 °C) and acidic (pH ~2) edge of a hot spring within a solfatara on the geothermally active island of Lihir in Papua New Guinea. Sample material was stored for 3 days without temperature control during transfer to the laboratory. Microbial enrichments were performed in 250-ml Erlenmeyer flasks with 70 ml basal medium (Plumb *et al.*, 2002), which contained (per litre) 1.5 g (NH₄)₂SO₄, 0.25 g KH₂PO₄, 0.25 g MgSO₄·7H₂O and 0.1 g yeast extract, adjusted to pH 0.8 with 18 M H₂SO₄, supplemented with 1 % (w/v) chalcopryrite concentrate (88 % CuFeS₂, 4 % FeS₂ and trace quantities of SiO₂, FeS and clinichlore) with a mean particle size of 37.5 µm (P₈₀=85 µm) and 1 % (w/v) sulfidic ore material collected from Lihir. Enrichment cultures were incubated at 72 °C in a shaker incubator at 150 r.p.m. After incubation for 1 week, abundant growth of small, irregular-shaped cocci was detected by using a phase-contrast microscope (Leitz Diaplan). The enrichment culture was subcultured into fresh medium of the same composition once and then maintained on growth medium

with 1 % (w/v) chalcopyrite concentrate as the energy source. A pure strain, designated JP7^T, was isolated by using serial decimal dilutions to extinction in shake flasks of basal medium at pH 0.8 supplemented with 1 % (w/v) chalcopyrite concentrate. Strain JP7^T was maintained routinely in shake flasks with this medium and the incubation conditions given above. Unless specified otherwise, strain JP7^T was grown in basal medium at pH 0.8. Cell morphology was similar to other members of the order *Sulfolobales*, with slightly aspherical shape, and occasional planar sides and edges. Cells occurred singly or occasionally in pairs, the latter particularly during the exponential growth phase. On all growth media, cells ranged between 0.5 and 1.5 µm in diameter and were non-motile. Cells of strain JP7^T stained Gram-negative.

Energy sources for growth of strain JP7^T were tested in shake flask culture incubated aerobically using the basal medium described above. Chemically defined inorganic substrates tested were elemental sulfur (5 g l⁻¹) and FeSO₄·7H₂O (10 g l⁻¹). A stock solution of FeSO₄·7H₂O was sterilized by filtration through a 0.2-µm pore size filter. Elemental sulfur was sterilized by heating at 100 °C for 1 h, which was repeated twice on consecutive days. The concentration of Fe²⁺ was determined spectrophotometrically (Wilson, 1960). Growth on elemental sulfur was determined by phase-contrast microscopy and by measuring total solution sulfur concentration by using inductively coupled plasma optical emission spectroscopy. Although specific detection of sulfate was not performed, it was assumed that elemental sulfur was oxidized completely to sulfate as the pH of the medium decreased significantly, presumably because of production of sulfuric acid. Strain JP7^T grew successfully using Fe²⁺ or sulfur as an energy source, although to lower cell density than growth on chalcopyrite concentrate. Strain JP7^T oxidized Fe²⁺ rapidly compared with uninoculated controls; however, growth using Fe²⁺ as the sole energy source was not sustained in subsequent subcultures without the addition of elemental sulfur or mineral sulfide or other reduced sulfur compounds. Complex mineral sulfides were also tested as energy sources for growth of strain JP7^T. These tests were conducted in shake flasks containing basal medium at pH 0.8. Mineral sulfides tested included pyrite (1 %, w/v), chalcopyrite and arsenopyrite (1–10 %, w/v), which were added as sterilized, finely ground concentrates or finely ground massive sulfides. Growth was monitored with a phase-contrast microscope over three subsequent subcultures where possible. Strain JP7^T grew well on all three mineral sulfide substrates and maintained good growth over numerous subcultures on chalcopyrite and arsenopyrite up to a loading of 10 % (w/v). Growth was also observed on chalcopyrite and pyrite in basal medium prepared without yeast extract at pH 0.8 over subsequent subcultures, with a cell density of 10⁸ cells ml⁻¹ achieved after 5–7 days incubation. A significantly higher growth rate on chalcopyrite was achieved when the growth medium contained yeast extract, with a cell density of 10⁸ cells ml⁻¹ achieved after 3 days incubation. These results suggested that strain

JP7^T is a facultative autotroph, although confirmation of this via uptake of radio-labelled carbon was not performed. Optimal growth was achieved in medium containing NaCl up to a concentration of 0.5 % (w/v). No growth occurred in medium containing NaCl at a concentration of 1 % (w/v). Strain JP7^T was less halotolerant than previously described species of the genus *Acidianus*, which are able to grow in medium containing up to 4 % (w/v) NaCl (Seeger *et al.*, 1986).

Growth of strain JP7^T under anaerobic conditions was tested by using H₂ or H₂S (Na₂S·9H₂O added at 2 g l⁻¹) as an electron donor and elemental sulfur or ferric iron [Fe₂(SO₄)₃ at 10 g l⁻¹] as a terminal electron acceptor. *A. infernus* DSM 3191^T, *A. ambivalens* DSM 3772^T and *A. brierleyi* DSM 1651^T were included as reference strains in anaerobic growth tests. The media and growth conditions used were as described by Seeger *et al.* (1986) except that no sodium sulfide was added to the anaerobic media. All cultures showing growth were subcultured twice for confirmation of growth. *A. infernus* DSM 3191^T and *A. ambivalens* DSM 3772^T grew by oxidizing H₂ and by reducing elemental sulfur to H₂S as expected. *A. brierleyi* DSM 1651^T and strain JP7^T did not grow by oxidizing H₂ or by reducing elemental sulfur. *A. brierleyi* has previously been reported to grow by oxidizing H₂ and by reducing elemental sulfur (Seeger *et al.*, 1986), although, as discussed below, interpretation of this result is difficult owing to the addition of sodium sulfide (0.75 g l⁻¹) to the anaerobic growth medium. Strain JP7^T and *A. brierleyi* grew by oxidizing H₂S and by reducing ferric iron (Fig. 1) both with and without the addition of organic carbon. Strain JP7^T and *A. brierleyi* DSM 1651^T grew when sodium sulfide was added at 0.75 g l⁻¹, the concentration used in the anaerobic media prepared by Seeger *et al.* (1986). This result suggests that the previously reported growth by *A.*

Fig. 1. Cell densities (solid symbols) and ferrous iron concentration (open symbols) for growth of strain JP7^T (triangles) and *A. brierleyi* DSM 1651^T (squares) in anaerobic medium by oxidation of H₂S and reduction of ferric iron. Reduction of ferric iron to ferrous iron in an uninoculated control (open circles) is also shown. No cells were detected in the uninoculated control.

brierleyi with H_2 as an energy source may have been due to the oxidation of H_2S produced as a result of the addition of sodium sulfide to the anaerobic medium. *A. infernus* DSM 3191^T and *A. ambivalens* DSM 3772^T did not grow via this redox couple. None of the four strains grew in anaerobic medium by oxidizing H_2 and reducing ferric iron, in contrast to the results for '*A. manzaensis*' (Yoshida *et al.*, 2006). Brock & Gustafson (1976) showed that *Sulfolobus acidocaldarius* grew by oxidizing elemental sulfur or glutamate and by reducing ferric iron and, more recently, '*A. manzaensis*' was shown to be able to grow by oxidizing elemental sulfur or H_2 and by reducing ferric iron (Yoshida *et al.*, 2006). *A. brierleyi* and *A. infernus* grew by oxidizing elemental sulfur using molybdate as a terminal electron acceptor (Seeger *et al.*, 1986). All four tested strains grew in anaerobic medium by oxidizing H_2S and by reducing elemental sulfur. The inability of strain JP7^T to oxidize H_2 under sulfur-reducing or ferric-iron-reducing conditions suggests that this strain lacks the hydrogenase used by *A. ambivalens* for this metabolism (Laska *et al.*, 2003). A number of studies have identified the enzyme systems responsible for aerobic and anaerobic metabolism in *A. ambivalens* (reviewed by Kletzin *et al.*, 2004). More work is needed to characterize the mechanisms for anaerobic growth of other species of the genus *Acidianus*.

Aerobic organotrophic growth by strain JP7^T was tested by using glucose, sucrose, cellobiose, gelatin, yeast extract, meat extract and tryptone (each at 1 g l^{-1}), each provided separately as the sole energy source. Cell growth was monitored by phase-contrast microscopy. Basal medium containing $0.1\text{ g yeast extract l}^{-1}$ at pH 0.8 was used to provide a source of micronutrients, carbon or other growth requirements for testing of organic substrates. Strain JP7^T was unable to grow using any of the organic substrates as a sole energy source.

The temperature range for growth of strain JP7^T was determined via a temperature gradient incubator (Terratec Asia Pacific) and by application of the Ratkowsky equation (Ratkowsky *et al.*, 1983; Plumb *et al.*, 2002; Franzmann *et al.*, 2005). For this test, strain JP7^T was grown in a mineral-sulfide-free medium that enabled non-destructive turbidimetric measurement of growth. The medium contained (per litre) $0.4\text{ g MgSO}_4 \cdot 7H_2O$, $0.4\text{ g (NH}_4)_2\text{SO}_4$, $0.4\text{ g KH}_2\text{PO}_4$, $4.0\text{ g K}_2\text{S}_4\text{O}_6$, $20.0\text{ g FeSO}_4 \cdot 7H_2O$ and $1.0\text{ g meat extract}$, with pH adjusted to 0.8 with $18\text{ M H}_2\text{SO}_4$. The potassium tetrathionate, ferrous sulfate and meat extract were added as filter-sterilized solutions to the basal medium after sterilization by autoclaving at 121°C for 20 min. Biomass in cultures grown at 24 different temperatures ranging from 40 to 85°C was quantified by measuring the optical density at 550 nm . The square root of the inverse of the time taken to reach an optical density of 0.25 at each temperature was plotted against temperature and the plot was fitted by using the Ratkowsky equation. Extrapolated minimum, optimum and maximum temperatures for growth were 45.2 ± 1.3 , 74.4 ± 0.7 and $82.5 \pm 0.8^\circ\text{C}$, respectively. At the temperature closest to the extrapolated optimum at which strain JP7^T

was grown (72.9°C), the generation time of strain JP7^T in this mixotrophic medium was 8.4 h. Strain JP7^T was not as thermophilic as *A. infernus* or *A. ambivalens*, which grew optimally at 90 and 80°C , respectively (Seeger *et al.*, 1986; Zillig *et al.*, 1986), but was more thermophilic than *A. brierleyi*, which grew optimally at 70°C (Seeger *et al.*, 1986).

The pH range for growth of strain JP7^T was determined by using two different approaches. Growth at different pH was tested with either basal medium containing chalcophyrite concentrate (1% , w/v) or basal medium supplemented with $\text{FeSO}_4 \cdot 7H_2O$ (10 g l^{-1}). The pH of the medium was adjusted by using concentrated H_2SO_4 . All pH measurements were made at room temperature by using a SenTix HW electrode and a pH 330i meter (WTW). Standard solutions of pH 2.0 and pH 1.0 buffer (Merck) were used for calibration and to test the accuracy of low pH readings. Inoculated and uninoculated control flasks were set up in duplicate. As stated above, strain JP7^T required a small amount of sulfur or mineral sulfide in the growth medium in order to maintain successful growth over repeated subcultures; however, the inclusion of sulfur or mineral sulfide causes significant changes in pH of the medium owing either to acid production via sulfur oxidation or to acid consumption via mineral sulfide oxidation, and the resulting change in pH makes assessment of the pH range for growth difficult. In order to minimize changes in pH of the medium due to addition of sulfur or mineral sulfide, the effect of pH on the growth of strain JP7^T was tested in basal medium containing Fe^{2+} as the sole energy source. Inoculum was prepared by growing strain JP7^T in basal medium at pH 0.8 or 1.8 with chalcophyrite concentrate (1% , w/v). All flasks were incubated in a shaker operated at $\sim 72^\circ\text{C}$, with shaking at 150 r.p.m. Cultures grown on chalcophyrite were monitored by using a Helber counting chamber and phase-contrast microscopy. In cultures grown on ferrous sulfate, oxidation of Fe^{2+} was monitored by measuring the Fe^{2+} concentration spectrophotometrically (Wilson, 1960). Strain JP7^T grew and oxidized Fe^{2+} over a broad range of pH. At the extremes of the pH range tested (pH 0.4 and 3.0) strain JP7^T oxidized the least Fe^{2+} (Fig. 2a). Strain JP7^T oxidized Fe^{2+} at a similar rate at pH 0.8, 1.0 and 1.4, although a slight lag prior to achieving the maximum oxidation rate was observed at pH 0.8 and 1.0. Slower rates of Fe^{2+} oxidation were observed at other test pH values, but rates of Fe^{2+} oxidation at these pH values were greater than occurred in the uninoculated controls. Growth on chalcophyrite concentrate was observed at all pH values tested up to a pH of 2.2. A comparison of the growth of strain JP7^T on chalcophyrite over a range from pH 0.4 to 1.0 was made (Fig. 2b). At such a low pH, acid production or consumption by the mineral sulfide does not change pH effectively. Growth at pH 0.4, 0.6 and 0.8 occurred after an initial lag period; however, the growth rate and maximum cell density achieved at pH 0.8 and 1.0 were almost identical. In another test, cell growth was maintained over numerous subcultures in basal medium with chalcophyrite (1% , w/v) at pH 0.35. At this extremely low pH, cells remained intact but were

Fig. 2. Plot showing the relationship between growth and pH for strain JP7^T based on oxidation of Fe²⁺ (a) and growth on chalcopyrite concentrate (b). Uninoc., Uninoculated.

smaller in size and slightly refractile in appearance, probably because of their stressed condition. The pH range for growth of strain JP7^T extended to significantly lower values than for other species of *Acidianus*. According to Segerer *et al.* (1986) and Zillig *et al.* (1986), the lower pH limit for growth of *A. infernus*, *A. ambivalens* and *A. brierleyi* was pH 1.0, with optimal growth occurring between pH 1.5 and 2.0.

The 16S rRNA gene was amplified from cells of strain JP7^T by using direct lysis PCR with primers 25Fa (5'-TCYG-GTTGATCCYGCCRG-3') and 1492R (5'-ACGGITACC-TTGTTACGACTT-3'). A 1 ml sample of culture was centrifuged at 12 000 g for 8 min to pellet the cells. Pelleted cells were resuspended in 1 ml PBS (pH 7.2) and 1 µl of this solution was then used as template for PCR by using the HotstartTaq Master Mix (Qiagen). The PCR products were purified with the UltraClean PCR Clean-up kit (MO BIO Laboratories Inc.) and both strands were sequenced by using the BigDye Terminator version 3.1 cycle-sequencing kit (Applied Biosystems) and primers 25Fa, 530Fa (5'-GTGTCAGCCGCCGCGG-3'), 934Fa (5'-AGGAATTGCGGGGGAGCAG-3'), 519R (5'-TTACCG-CGGCGGCTG-3'), 915R (5'-GTGCTCCCCGCCAATT-CCT-3') and 1492R. A near-complete 16S rRNA gene contiguous sequence (1473 bases) was assembled by using

Chromas Pro (Technelysium) and then compared with sequence data in the GenBank database via BLAST (Altschul *et al.*, 1997) available at <http://www.ncbi.nlm.nih.gov>. The sequence was then aligned against reference sequences of species of *Acidianus* by using the program ARB (Ludwig *et al.*, 2004). A phylogenetic tree was constructed by using distance matrix calculations and the neighbour-joining method (Saitou & Nei, 1987) available in the ARB program. The stability of tree nodes was evaluated by using bootstrap resampling (Felsenstein, 1985). Construction of a similarity matrix was performed also within ARB by using representative sequences from the *Sulfolobales*. The 16S rRNA gene sequence of strain JP7^T was most closely related to that of '*A. manzaensis*' NA-1, with a similarity of 97.2 %. The 16S rRNA gene sequences of *A. ambivalens* DSM 3772^T, *A. infernus* DSM 3191^T and *A. brierleyi* DSM 1651^T shared similarity of 92.6, 92.3 and 91.2 %, respectively, with that of strain JP7^T. On the basis of 16S rRNA gene sequence analysis, there is probably some doubt as to whether *A. brierleyi* should be included in the genus *Acidianus*, although it shares many phenotypic similarities with other members of this genus. Similarity of the 16S rRNA gene sequence of strain JP7^T to those of the type strains of two other representatives of the *Sulfolobaceae*, *Metallosphaera prunae* and *Sulfolobus solfataricus*, was 90 and 87.7 %, respectively. A phylogenetic tree generated from 16S rRNA gene sequence data from members of the *Sulfolobales* (Fig. 3) shows strain JP7^T clustering with '*A. manzaensis*'. The positioning of strain JP7^T with other species of the genus *Acidianus* was well supported according to bootstrap analysis.

Genomic DNA for the determination of base composition was extracted from a late-exponential phase culture according to the method of Plumb *et al.* (2001), but without the use of the bead-beating step. The DNA was purified by using the UltraClean PCR Clean-up kit (MO BIO Laboratories Inc.). Agarose gel electrophoresis was used to confirm the extraction of high-molecular-mass genomic DNA. The DNA was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989). The G + C content was determined by analysing the resultant deoxyribonucleosides by HPLC (Shimadzu Corp.). The column used for analysis was a VYDAC 201SP54 (C₁₈, 5 µm, 250 × 4.6 mm) equipped with a 201GD54H guard column (Vydac). The DNA G + C content of strain JP7^T was 31.1 mol%, which is close to that of *A. ambivalens* (31 mol%), *A. infernus* (32.7 mol%) and *A. brierleyi* (31.5 mol%) (Segerer *et al.*, 1986; Zillig *et al.*, 1986).

Freeze-dried biomass collected from cultures of strain JP7^T was used to characterize the cell membrane phospholipids. Biomass was placed in a thick-walled test tube along with 3 ml of a mixture containing 10:1:1 methanol/CHCl₃/concentrated HCl. The test tube was capped and the mixture was heated overnight at 95 °C in a water bath. After cooling, 1 ml water was added and the reaction mixture was extracted three times with 1.8 ml portions of a 4:1 mixture of n-hexane and CH₂Cl₂. The extracts were pooled and the

Fig. 3. Phylogenetic tree based on 16S rRNA gene sequence data generated by using distance matrix and neighbour-joining methods for selected members of the *Sulfolobales* (GenBank accession numbers are given in parentheses) showing the affiliation of strain JP7^T with members of the genus *Acidianus*. Bootstrap values are percentages based on 1000 resamplings. Bar, 10% sequence divergence.

solvent was removed under a stream of N₂. The ether lipids were stored at -20 °C. Gas chromatographic analysis was carried out by using a Hewlett Packard 5890 gas chromatograph equipped with an on-column auto-injector and a flame-ionization detector that was maintained at 405 °C. The capillary column used was a 2.5-m polyimide-clad SGE HT5, with an internal diameter of 0.25 mm and a film thickness of 0.1 µm (SGE International). Samples were injected with the injector and column at 30 °C. The oven temperature was maintained for 2 min, then increased at 35 °C min⁻¹ to 350 °C and then at 5 °C min⁻¹ to 400 °C. At the end of the run, the oven was cooled at a rate of 30 °C min⁻¹ in order to reduce the likelihood of column damage as a result of rapid temperature change. The carrier gas was helium and the head pressure was 35 kPa. The ether lipids eluted as well-separated peaks. The concentrations of glycerol dialkyl glycerol tetraethers (GDGTs) and glycerol dialkyl calditol tetraethers (calditols) were determined. Analysis of the ether lipids in strain JP7^T showed an almost 50 : 50 split between GDGTs and calditols. Cyclized forms of each of these tetraethers with one to eight mid-chain cyclopentyl rings were detected, with the majority of tetraethers containing six rings. Although the distribution of cyclized tetraether lipids varies according to factors such as temperature and pH, this ether lipid profile was consistent with profiles of members of the *Sulfolobales* grown at high temperature (De Rosa *et al.*, 1983).

The phenotypic and genotypic characteristics described above suggest that strain JP7^T is a member of the genus *Acidianus*. Differences in 16S rRNA gene sequence, aerobic and anaerobic growth, temperature range for growth and pH range for growth justify the conclusion that strain JP7^T represents a novel species of the genus *Acidianus*. The name *Acidianus sulfidivorans* sp. nov. is proposed for this novel species.

Emended description of *Acidianus* Segerer *et al.* 1986

Cells are Gram-negative and occur singly or occasionally in pairs during exponential growth; the cells have an irregular

coccoid morphology (sometimes lobed or showing sharp bends, and resembling tetrahedrons, pyramids, discs or dishes). Cells are non-motile. Cell width is between 0.5 and 2 µm, depending on culture conditions. A surrounding envelope about 25 nm wide covering the cell membrane is evident in thin section. The envelope is composed of subunits in a hexagonal array. The cells are facultative aerobes. Lithotrophic growth occurs aerobically by means of S⁰ oxidation, mineral sulfide oxidation or Fe²⁺ oxidation. Anaerobic growth occurs by means of S⁰ or ferric iron reduction with H₂ or H₂S. The cells are autotrophic and mixotrophic and may grow organotrophically on yeast extract without S⁰ in the presence of O₂. The organisms are thermoacidophiles, thriving between pH 0.35 and 6 and between 45 and 96 °C. They grow in the presence of 0.1% NaCl and may grow in media containing up to 4% NaCl. Neither muramic acid nor *meso*-diaminopimelic acid is present, indicating the absence of murein. Elongation factor 2 is sensitive to adenosine diphosphate ribosylation by diphtheria toxin. Cells contain isopranyl ether lipids, which consist of GDGTs and calditols, including lipids with mid-chain cyclopentyl rings, and also caldariellaquinone. Members of the genus are resistant to vancomycin, ampicillin and kanamycin (150 µg ml⁻¹ each). DNA-dependent RNA polymerase shows incomplete immunological cross-reactions with antibody against DNA-dependent RNA polymerase from *Sulfolobus acidocaldarius* DSM 639^T. The purified DNA has a G + C content of 31–33 mol%. Members of the genus occur in acidic solfataras and in marine hydrothermal systems. The type species is *Acidianus infernus*.

Description of *Acidianus sulfidivorans* sp. nov.

Acidianus sulfidivorans (sul.fi.di.vo'rans. N.L. n. *sulfidum* sulfide; L. part. adj. *vorans* devouring; N.L. part. adj. *sulfidivorans* sulfide-devouring).

Displays the following properties in addition to those given in the emended genus description. Cells are 0.5–1.5 µm in diameter. Growth is obligately chemolithotrophic. Good

growth occurs under aerobic conditions on elemental sulfur, ferrous iron, pyrite and other mineral sulfides. Under anaerobic conditions, ferric iron or elemental sulfur serve as the terminal electron acceptor for growth via oxidation of H₂S. Cells do not grow using elemental sulfur as a terminal electron acceptor for oxidation of H₂. Cells are facultatively autotrophic, as they grow when provided with CO₂ as the sole source of carbon and heterotrophically using yeast extract or meat extract as a carbon source. Cells do not grow organotrophically. Cells grow over a range of temperatures from 45 to 83 °C, with optimum growth at 74 °C. The pH range for growth is 0.35–3.0, with optimal growth at pH 0.8–1.4. Optimal growth occurs at up to 0.5 % (w/v) NaCl. The DNA G+C content is 31.1 mol%. Cell-membrane lipids consist of a mixture of GDGTs and glycerol dialkyl calditol tetraethers, most of which are cyclized tetraethers.

The type strain, JP7^T (=DSM 18786^T=JCM 13667^T), was isolated from the sulfur-rich acidic edge of a solfataric hot spring.

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