**Archeoglobus infectus** sp. nov., a novel thermophilic, chemolithoheterotrophic archaeon isolated from a deep-sea rock collected at Suiyo Seamount, Izu-Bonin Arc, western Pacific Ocean

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A novel thermophilic, strictly anaerobic archaeon, designated strain Arc51T, was isolated from a rock sample collected from a deep-sea hydrothermal field in Suiyo Seamount, Izu-Bonin Arc, western Pacific Ocean. Cells of the isolate were irregular cocci with single flagella and exhibited blue-green fluorescence at 436 nm. The optimum temperature, pH and NaCl concentration for growth were 70°C, pH 6.5 and 3% (w/v), respectively. Strain Arc51T could grow on thiosulfate or sulfite as an electron acceptor in the presence of hydrogen. This strain required acetate as a carbon source for its growth, suggesting that the reductive acetyl CoA pathway for CO2 fixation was incomplete. In addition, coenzyme M (2-mercaptoethanesulfonic acid), which is a known methyl carrier in methanogenesis, was also a requirement for growth of the strain. Analysis of the 16S rRNA gene sequence revealed that the isolate was similar to members of the genus Archeoglobus, with sequence similarities of 93.6–97.2%; the closest relative was Archeoglobus veneficus. Phylogenetic analyses of the dsrAB and apsA genes, encoding the alpha and beta subunits of dissimilatory sulfite reductase and the alpha subunit of adenosine-5’-phosphosulfate reductase, respectively, produced results similar to those inferred from comparisons based on the 16S rRNA gene sequence. On the basis of phenotypic and phylogenetic data, strain Arc51T represents a novel species of the genus Archeoglobus, for which the name Archeoglobus infectus sp. nov. is proposed. The type strain is Arc51T (=NBRC 100649T = DSM 18877T).

Energy acquisition from dissimilatory sulfate reduction is widespread among prokaryotes. The ability to reduce sulfate is found not only in members of the domain Bacteria, but also in the domain Archaea. Archeoglobus fulgidus, which belongs to the phylum Euryarchaeota, was the first sulfate-reducing archaeon isolated from the hot sediment of a hydrothermal vent system in Italy (Stetter et al., 1987). Following this discovery, Caldivirga maquilensis, a hyperthermophilic and acidophilic sulfate reducer belonging to the phylum Crenarchaeota, was isolated from a hot spring in the Philippines (Itoh et al., 1999).

In the phylum Euryarchaeota, only three sulfate (or sulfite)-reducing species in the genus Archeoglobus have validly published names thus far: A. fulgidus (Stetter et al., 1987; Stetter, 1988), Archeoglobus profundus (Burggraf et al., 1990) and Archeoglobus veneficus (Huber et al., 1997). All of these species were isolated from a hydrothermal vent system. In addition, there is a species that currently lacks a validly published name, namely ‘Archeoglobus lithothrophicus’ (Stetter et al., 1993); this species was investigated with regard to its autotrophic CO2 fixation (Vorholt et al., 1995). Whilst A. fulgidus, A. profundus and ‘A. lithothrophicus’ are true sulfate reducers that can utilize sulfate as an electron acceptor, A. veneficus cannot reduce sulfate, but utilizes thiosulfate or sulfite as an electron sink. The related genera Ferroglobus (Hafenbradl et al., 1996) and Geoglobus (Kashefi et al., 2002), which show no ability to reduce...
sulfate and/or sulfite, also belong to the family Archaeoglobaceae, along with the genus *Archaeoglobus*. *Ferroglobus placidus* grows anaerobically by using nitrate as an electron acceptor in the presence of Fe(II) as an electron donor. It is also capable of utilizing thiosulfate and Fe(III) as electron acceptors (Hafenbradl *et al.*, 1996; Tor *et al.*, 2001). *Geoglobus alhangari* can grow in the presence of Fe(III) as the sole electron acceptor and does not utilize any other acceptors, such as sulfur compounds (Kashefi *et al.*, 2002). All species belonging to the family Archaeoglobaceae have been isolated from hydrothermal vent systems and demonstrate anaerobic growth involving the use of sulfur compounds and/or Fe(III) as electron sinks.

Recently, we isolated a novel thermophilic, strictly anaerobic archaeon, showing sulfite/thiosulfate reduction, from a hydrothermal rock sample collected from Suiyo Seamount. Data from phylogenetic and phenotypic analyses described in this paper demonstrated that this isolate warrants classification as a novel species in the genus *Archaeoglobus*.

Suiyo Seamount in Izu-Bonin Arc, western Pacific Ocean (28° 34′ N 140° 29′ E; depth, 1380 m) is an active submarine volcano with many active hydrothermal vents in its caldera (Glasby *et al.*, 2000). A black smoker chimney (at approx. 300 °C) is observed frequently and large amounts of reduced chemicals have been detected in the hydrothermal fluids discharged from the vents (Tsunogai *et al.*, 1994). Recently, an archaela community in the site was investigated by using 16S rRNA gene-clone analyses: the data suggested that a novel *Archaeoglobus* species was an inhabitant (Hara *et al.*, 2005; Higashi *et al.*, 2004). A thermophilic sulfite/thiosulfate-reducing archaeon, designated strain Arc51T, was isolated from a rock sample collected on 23 July 2002 by using a benthic multi-coring system (Metal Mining Agency of Japan, Tokyo, Japan), which is a tethered marine rock drill used to obtain cores from the seabed.

A core sample was collected from near the chimneys. Some rocks at various depths (to a maximum of 7035 mm from the seafloor) were selected for enrichment, crushed immediately in a vice in an anaerobic chamber (COY Laboratory Products) and resuspended in a basal medium. The basal medium (pH 6.5 at room temperature), kept under an N2/CO2 (80 : 20, v/v) atmosphere, comprised the following salts and solutions (L⁻¹): KH₂PO₄, 0.60 g; K₂HPO₄, 0.11 g; MgCl₂.6H₂O, 0.75 g; CaCl₂.2H₂O, 0.15 g; Fe(NH₄)₂(SO₄)₂, 2 mg; NH₄Cl, 0.54 g; NaCl, 30 g; Na₂CO₃, 1 g; trace element solution [comprising the following (L⁻¹): disodium EDTA, 0.64 g; MgSO₄.7H₂O, 6.2 g; MnSO₄.4H₂O, 0.55 g; NaCl, 1 g; FeSO₄.7H₂O, 0.1 g; CoCl₂.6H₂O, 0.17 g; CaCl₂.2H₂O, 0.13 g; ZnSO₄.7H₂O, 0.18 g; CuSO₄.5H₂O, 0.05 g; KAI(SO₄)₂.12H₂O, 0.018 g; H₂BO₃, 0.01 g; Na₂MoO₄.2H₂O, 0.011 g; NaCl, 0.062 g; NaOH, 0.025 g; NBRC, 2005], 2 ml; vitamin solution [comprising the following (L⁻¹): biotin, 2 mg; folic acid, 2 mg; pyridoxine/HCl, 10 mg; thiamine/HCl, 5 mg; riboflavin, 5 mg; nicotinic acid, 5 mg; calcium pantothenate, 5 mg; p-aminobenzoic acid, 1 mg; vitamin B12, 0.01 mg; NBRC, 2005], 2 ml; resazurin, 1 mg; Na₂S₉H₂O, 0.35 g. Enrichment and isolation were performed under an H₂/CO₂ atmosphere (80 : 20, v/v; 150 kPa) using vials sealed with butyl-rubber stoppers and aluminium caps, and the medium used was a basal medium supplemented with Na₂SO₄ (1.42 g L⁻¹), Na₂S₂O₃.5H₂O (2.48 g L⁻¹) and sodium acetate (0.82 g L⁻¹) under an H₂/CO₂ atmosphere (80 : 20, v/v; 150 kPa). After a 1 week incubation at 75 °C, microbial growth was observed in a culture that had been inoculated with the surface layer of the core sample. The enrichment culture was transferred to a fresh enrichment medium (inoculum: 10%, v/v) and this procedure was repeated several times. After 2 weeks cultivation at 75 °C on the isolation medium solidified with 0.6% gellan gum, a single black colony had formed. After a second purification step with the same solid medium, strain Arc51T was isolated successfully from the enrichment sample. However, the isolate showed very slow growth and, after a while, the growth came to a complete standstill. After some trial and error, it was finally found that supplementation with coenzyme M, a methyl carrier essential for methanogenesis, restored growth. Subsequently, cultivations were performed using medium supplemented with coenzyme M at 0.14 g L⁻¹ (final concentration).

Morphologically, cells of strain Arc51T were irregular cocci (approx. 0.5–1.0 μm in diameter) with single flagella (Fig. 1). Microscopically, strain Arc51T showed blue-green...
fluorescence at 436 nm; this suggested that it possessed a coenzyme F$_{420}$-like compound. The isolate was a strictly anaerobic archaeon and could grow with thiosulfate under an H$_2$/CO$_2$ atmosphere. It required acetate as a carbon source, and growth was not observed after 7 days incubation with the addition of 1% elemental sulfur. Analysis of the gas phase of the cultures by GC performed using a thermal-conductivity detector and a Molecular Sieve 60/80 column (both from Shimadzu) revealed that strain Arc51$^T$, like A. fulgidus and A. veneficus, produced small amounts of methane (approx. 85 nmol from a 20 ml culture).

After the extraction and purification of genomic DNA (Mori et al., 2000), the 16S rRNA gene was amplified with primers 25e (forward) and 1525 (reverse) (Achenbach & Woese, 1995). The PCR product was sequenced by using the PCR primers as well as the following: Ar700F, 5'-ACTACCCGGGTATCTAATC-3'; Ar1000F, 5'-AGTCA-GGCAACGAGCGAGA-3'; Ar1000R, 5'-TCTCGCTCGTTGCCTGACT-3' (Hattori et al., 2000). An almost-complete 16S rRNA gene sequence was determined for strain Arc51$^T$ (GenBank accession no. AB274307). After alignment with the ARB program (Ludwig et al., 2004), a phylogenetic tree (Fig. 2a) was constructed by using the neighbour-joining method with the CLUSTAL_X program (Saitou & Nei, 1987; Thompson et al., 1997). Strain Arc51$^T$ belonged to the family Archaeoglobaceae, and sequence similarities between the isolate and A. veneficus, A. fulgidus, A. profundus, G. ahangari and F. placidus were 97.2, 95.7, 93.6, 95.4 and 94.5%, respectively.

In addition to the 16S rRNA gene sequence, the dsrAB (encoding the alpha and beta subunits of dissimilatory sulfite reductase) and apaA (encoding the alpha subunit of adenosine-5'-phosphosulfate reductase) gene sequences of strain Arc51$^T$ were determined. The dsrAB and apaA genes were amplified by using primers DSR1F and DSR4R (Wagner et al., 1998) and primers APS7-F and APS8-R (Friedrich, 2002), respectively. Amino acid sequences were deduced from the gene sequences determined for dsrAB (GenBank accession no. AB274309, 1870 bp) and apaA (GenBank accession no. AB274310, 889 bp) of strain Arc51$^T$, and neighbour-joining trees were constructed with the CLUSTAL_X program. The dsrAB gene sequence of A. veneficus DSM 11195$^T$ was also determined by the same method (GenBank accession no. AB274311, 1870 bp) and used for phylogenetic analysis. The phylogenetic trees for DsrAB (Fig. 2b) and ApsA (Fig. 2c) were topologically similar to that for the 16S rRNA gene, and the closest relative in each tree was A. veneficus, with similarities of 78.3 and 92.8% for the DsrAB and ApsA sequences, respectively.

A test for determining the electron acceptors utilized was performed in the presence of acetate (10 mM) under an atmosphere of H$_2$/CO$_2$.
H$_2$/CO$_2$ atmosphere. Growth was examined by using microscopic observation and changes in OD$_{660}$ recorded with a spectrophotometer (model U-2800; Hitachi). The concentrations of sulfate, thiosulfate and nitrate were determined by using HPLC apparatus (model 2695; Waters) equipped with a Waters I.C.-Pac anion column and a Waters (model 432) conductivity detector (column temperature, 35°C; eluant, sodium gluconate at 0.32 g l$^{-1}$, boric acid at 0.36 g l$^{-1}$, sodium tetraborate decahydrate at 0.5 g l$^{-1}$, butanol at 20 ml l$^{-1}$ and acetonitrile at 120 ml l$^{-1}$; flow rate, 1.3 ml min$^{-1}$). Strain Arc51$^T$ could utilize thiosulfate (10 mM) and sulfite (2.5 mM) as electron acceptors, but the following acceptors did not support growth: sulfate (10 mM), elemental sulfur (10 g l$^{-1}$), nitrate (10 mM), nitrite (2.5 and 5.0 mM), oxygen (5%), DMSO (2.5 and 5.0 mM), Fe(III) citrate (2.5 and 5.0 mM) and Fe(III) chloride (2.5 and 5.0 mM). Huber et al. (1997) reported that A. veneficus could not utilize nitrate or nitrite as electron acceptors. Our study revealed that, like A. veneficus, A. fulgidus NBRC 100126$^T$ (=VC-16$^T$) and A. profundus NBRC 100127$^T$ (=AV18$^T$) could not utilize these compounds.

In the presence of thiosulfate (20 mM) and acetate (10 mM), strain Arc51$^T$ grew on hydrogen as an electron donor. Under an N$_2$/CO$_2$ atmosphere, neither an increase in cell density nor a decrease in thiosulfate (20 mM) was observed by culturing the strain in a medium supplemented with the following substrates: acetate (20 and 40 mM), glucose (10 mM), butyrate (20 mM), citrate (20 mM), formate (20 and 40 mM), fumarate (20 mM), glutamate (20 mM), lactate (20 mM), malate (20 mM), propionate (20 mM), pyruvate (20 mM), succinate (20 mM), L-arginine (20 mM), L-asparagine (20 mM), L-cysteine (20 mM), L-histidine (20 mM), L-leucine (20 mM), L-methionine (20 mM), ethanol (20 mM), 2-propanol (20 mM), methanol (20 mM), Bacto peptone (Difco) (0.5 g l$^{-1}$), Bacto yeast extract (Difco) (0.5 g l$^{-1}$) and Casamino acids (0.5 g l$^{-1}$). The results indicated clearly that strain Arc51$^T$ was an obligate chemolithotroph and incapable of heterotrophic fermentation. Furthermore, in the presence of nitrate as an electron acceptor, neither growth nor nitrate reduction (measured by HPLC) was observed with 20 mM FeS alone or FeS and H$_2$ (as H$_2}$/CO$_2$) as electron donor(s). Although F. placidus can grow under the above-mentioned conditions (Hafenbradl et al., 1996), it was ascertained in our study that no other Archaeoglobus species could grow under identical conditions.

The temperature, pH (adjusted by adding Na$_2$CO$_3$ at room temperature) and NaCl concentration ranges for growth were determined on the basis of increases in OD$_{660}$ and reductions in thiosulfate following a 2 week incubation using basal medium supplemented with thiosulfate (20 mM) and sodium acetate (10 mM) under an H$_2}$/CO$_2$ atmosphere. Strain Arc51$^T$ could grow between 60 and 75°C, the optimum temperature being 70°C. It was very sensitive to pH changes, and growth occurred only at pH 6.5–7.0 (optimum, pH 6.5). The NaCl concentrations for growth ranged from 1 to 4% (w/v), with an optimum of 3%. Strain Arc51$^T$ had a doubling time of 23.3 h under optimum growth conditions [70°C, pH 6.5 and at 3% (w/v) NaCl] and the final concentration of the cells measured after staining with DAPI was approximately 8 x 10$^6$ cells ml$^{-1}$.

The G+C content of genomic DNA of strain Arc51$^T$ was analysed by using HPLC with a reversed-phase column (Mesbah et al., 1989); the value obtained was 54.9 mol%. The values for reference species A. fulgidus NBRC 100126$^T$ (=VC-16$^T$), A. profundus NBRC 100127$^T$ (=AV18$^T$) and A. veneficus DSM 11195$^T$ (=SNP6$^T$) were also determined in this study by using the same method, as these values were previously determined by the $T_m$ method; the re-determined values were 47.6, 43.7 and 46.2 mol%, respectively. These values were slightly higher than those determined previously (Burggraf et al., 1990; Huber et al., 1997; Stetter et al., 1987).

The differential characteristics of strain Arc51$^T$ and strains of related species are summarized in Table 1. Strain Arc51$^T$ could grow by utilizing sulfur compounds for respiration; this feature is typical of members of the genus Archaeoglobus. All species of the family Archaeoglobaceae, except A. profundus, were able to grow chemolithoautotrophically. However, strain Arc51$^T$ required acetate as a carbon source for growth; A. profundus also exhibited this trait, requiring organic compounds such as acetate for its growth (Burggraf et al., 1990; Vorholt et al., 1995). However, the electron acceptors utilized by strain Arc51$^T$ were clearly different from those utilized by A. profundus. Although almost all Archaeoglobus species reduce sulfate, our isolate was unable to use sulfate as an electron acceptor; however, it was able to use thiosulfate or sulfite. The pattern was similar to that of A. veneficus (Huber et al., 1997). The optimal growth temperature for strain Arc51$^T$ was obviously lower than those of other species of the family Archaeoglobaceae; whilst other species in this family show optimal growth at, or above, 80°C, strain Arc51$^T$ was unable to grow at 80°C. The phylogenetic tree based on 16S rRNA gene sequences revealed that strain Arc51$^T$ belonged to the family Archaeoglobaceae (sequence similarities, 93.6–97.2%), with A. veneficus as the closest relative (sequence similarity, 97.2%). Similar trends were also found for phylogenetic analyses based on the dsrAB and ApS A amino acid sequences (Fig. 2b, c). Although all of the molecular phylogenetic analyses indicated that strain Arc51$^T$ was related closely to A. veneficus, there were significant differences between their sequences. The results from a DNA–DNA hybridization study (Tamaki et al., 2003) indicated that the degree of DNA relatedness between strain Arc51$^T$ and A. veneficus was <20% (Table 1), suggesting strongly that they should be considered as different species. Strain Arc51$^T$ was distinguished easily from A. veneficus by the following two phenotypic features. Firstly, although A. veneficus could grow chemolithoautotrophically in the

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Table 1. Characteristics of strain Arc51<sup>T</sup> and related strains in the family Archaeoglobaceae

Strains: 1, Arc51<sup>T</sup>; 2, A. fulgidus VC-16<sup>T</sup>; 3, A. profundus AV18<sup>T</sup>; 4, A. veneficus SNP6<sup>T</sup>; 5, G. ahangari 234<sup>T</sup>; 6, F. placidus AEDII12DO<sup>T</sup>. Data for related archaea were from Burggraf et al. (1990), Hafenbradl et al. (1996), Huber et al. (1997), Kashefi et al. (2002), Stetter et al. (1987), Stetter (1988), Tor et al. (2001), Vargas et al. (1998) and this study. +, Positive; –, negative; ND, not determined.

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<th>4</th>
<th>5</th>
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<td>Hot sediment of hydrothermal system, Vulcano, Italy</td>
<td>Deep-sea hydrothermal system, Guaymas, Mexico</td>
<td>Deep-sea wall of black smoker, Mid-Atlantic Ridge</td>
<td>Deep-sea hydrothermal system, Guaymas, Mexico</td>
<td>Sand/water of shallow beach, Vulcano, Italy</td>
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<td>–</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;/CO&lt;sub&gt;2&lt;/sub&gt; + S&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;; H&lt;sub&gt;2&lt;/sub&gt;/CO&lt;sub&gt;2&lt;/sub&gt; + 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;/CO&lt;sub&gt;2&lt;/sub&gt; + Fe&lt;sup&gt;3+&lt;/sup&gt; + H&lt;sub&gt;2&lt;/sub&gt;/CO&lt;sub&gt;2&lt;/sub&gt; + S&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2-&lt;/sup&gt;; H&lt;sub&gt;2&lt;/sub&gt;/CO&lt;sub&gt;2&lt;/sub&gt; + NO&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>5</td>
<td>4</td>
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*Indicated as the range.
†Data from this study.
presence of hydrogen and thiosulfate, the isolate was unable to sustain growth in the absence of acetate as a carbon source. Secondly, A. veneificus could grow at 80 °C, but the isolate was intolerant of this high temperature. On the basis of the data described above, therefore, strain Arc51T represents a novel species within the genus Archaeoglobus, for which the name Archaeoglobus infectus sp. nov. is proposed.

An interesting feature of strain Arc51T is that it requires the organic cofactor coenzyme M for growth. Coenzyme M is an essential terminal methyl carrier in methanogenesis (Graham & White, 2002; Taylor & Wolfe, 1974; Thauer, 1998) and promotes growth in some methanogenic members of the Archaea (Miller et al., 1986; Sprenger et al., 2000; Taylor et al., 1974). Coenzyme M has a role not only in the methanogenesis pathway in the Euryarchaeota, but also in the utilization of short-chain alkenes, as demonstrated in recent studies on propylene-, ethylene- and vinyl chloride-utilizing bacteria (Allen et al., 1999; Boyd et al., 2006; Coleman & Spain, 2003a, b; Danko et al., 2006; Mattes et al., 2005). Emission of trace amounts of methane is generally observed in the growing cultures of almost all Archaeoglobus species, but they are unable to grow by methanogenesis. Indeed, many enzymes involved in methanogenesis have been found in A. fulgidus (Klenk et al., 1997), A. profundus (Vorholt et al., 1995), ‘A. lithotrophicus’ (Vorholt et al., 1995) and even F. pladus (Vorholt et al., 1997), although whether the trace methanogenesis is fundamentally connected to their growth is open to investigation. Strain Arc51T has lost its ability to synthesize coenzyme M and requires it for growth, producing trace amounts of methane in the process. Assuming that the cofactor is necessary for methanogenesis in strain Arc51T, the methane production is of some relevance to its growth, as mainly supported by reduction of sulfur compounds. Whilst this suggests the need for further study, the significant relationship between methanogenesis and growth in this strain would be not limited to the isolate, but would be a feature of all Archaeoglobus species.

Description of Archaeoglobus infectus sp. nov.

Archaeoglobus infectus (in.fec’tus. L. masc. adj. infectus incomplete, pertaining to the requirement for acetate and coenzyme M for growth).

Cells are irregular cocci (0.5–1.0 μm in diameter) with single flagella and show blue–green fluorescence at 436 nm. Obligately anaerobic. Grows chemolithoheterotrophically by the oxidation of molecular hydrogen, using thiosulfate and sulfate as electron acceptors. Sulfate, elemental sulfur, nitrate, nitrite and Fe(III) are not used as electron acceptors. No organic compounds can be used as electron donors as a substitute for hydrogen. Acetate is required as a carbon source for growth. Coenzyme M is also necessary for growth. Elemental sulfur inhibits growth. Grows at 60–75 °C. Optimum growth occurs at 70 °C, pH 6.5 and 3 % (w/v) NaCl. The genomic DNA G+C content of the type strain is 46.0 mol% (as determined by HPLC).

The type strain, Arc51T (=NBRC 100649T=DSM 18877T), was isolated from a rock sample collected in a hydrothermal vent in Suiyo Seamount, Izu-Bonin Arc, western Pacific Ocean.

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References


