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

Koji Mori,^{1,2} Akihiko Maruyama,² Tetsuro Urabe,³ Ken-ichiro Suzuki¹ and Satoshi Hanada²

Correspondence

Koji Mori

mori@nbrc.nite.go.jp

¹NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation (NITE), 2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

²Institute for Biological Resources and Functions, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan

³Department of Earth and Planetary Science, University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan

A novel thermophilic, strictly anaerobic archaeon, designated strain Arc51^T, 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 Arc51^T 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 *Archaeoglobus*, with sequence similarities of 93.6–97.2%; the closest relative was *Archaeoglobus 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 Arc51^T represents a novel species of the genus *Archaeoglobus*, for which the name *Archaeoglobus infectus* sp. nov. is proposed. The type strain is Arc51^T (=NBRC 100649^T=DSM 18877^T).

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*. *Archaeoglobus 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 *Archaeoglobus* have validly published names thus far: *A. fulgidus* (Stetter *et al.*, 1987; Stetter, 1988), *Archaeoglobus profundus* (Burggraf *et al.*, 1990) and *Archaeoglobus 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 '*Archaeoglobus lithotrophicus*' (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. lithotrophicus*' 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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *dsrAB* and *apsA* gene sequences of strain Arc51^T are AB274307, AB274309 and AB274310, respectively, and that for the *dsrAB* gene sequence of *Archaeoglobus veneficus* is AB274311.

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 ahangari* 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 archaeal 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 Arc51^T, 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)₂SO₄, 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, 0.05 g; KAl(SO₄)₂·12H₂O, 0.018 g; H₃BO₃, 0.01 g; Na₂MoO₄·2H₂O, 0.011 g; NiCl₂·6H₂O, 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; Na₂S·9H₂O, 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 Arc51^T 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 Arc51^T were irregular cocci (approx. 0.5–1.0 µm in diameter) with single flagella (Fig. 1). Microscopically, strain Arc51^T showed blue–green

Fig. 1. Phase-contrast (a) and transmission electron (b) micrographs of cells of strain Arc51^T. Cells were negatively stained with 1% phosphotungstic acid (pH 7.0) and observed by using an electron microscope (model H-7600; Hitachi) operating at 100 kV. Bars: 4 µm (a); 0.5 µm (b).

fluorescence at 436 nm; this suggested that it possessed a coenzyme F₄₂₀-like compound. The isolate was a strictly anaerobic archaeon and could grow with thiosulfate under an H₂/CO₂ 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: Ar700R, 5'-ACTACCCGGGTATCTAATC-3'; Ar1000F, 5'-AGTCAGGCAACGAGCGAGA-3'; Ar1000R, 5'-TCTCGCTCGT-TGCCTGACT-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 *apsA* (encoding the alpha subunit of adenosine-5'-phosphosulfate reductase) gene sequences of strain Arc51^T were determined. The *dsrAB* and *apsA* 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 *apsA* (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

Fig. 2. Neighbour-joining phylogenetic trees based on 16S rRNA gene (a), DsrAB deduced amino acid (b) and ApsA deduced amino acid (c) sequences of strain Arc51^T and relatives. Numbers of alignment positions used for the analyses of the 16S rRNA gene, DsrAB and ApsA were 1156, 546 and 265, respectively. Bootstrap probabilities are indicated at branching points. GenBank accession numbers for reference sequences are shown in parentheses. Bars, 0.03 substitutions per compared nucleic acid (a); 0.03 substitutions per compared amino acid (b, c).

H₂/CO₂ atmosphere. Growth was examined by using microscopic observation and changes in OD₆₆₀ 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 IC-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 N₂/CO₂ 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 H₂ (as H₂/CO₂) 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 Na₂CO₃ at room temperature) and NaCl concentration ranges for growth were determined on the basis of increases in OD₆₆₀ and reductions in thiosulfate following a 2 week incubation using basal medium supplemented with thiosulfate (20 mM) and sodium acetate (10 mM) under an H₂/CO₂ 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 × 10⁶ 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 45.9 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 redetermined 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 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 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 ApsA 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

Table 1. Characteristics of strain Arc51^T and related strains in the family *Archaeoglobaceae*

Strains: 1, Arc51^T; 2, *A. fulgidus* VC-16^T; 3, *A. profundus* AV18^T; 4, *A. veneficus* SNP6^T; 5, *G. ahangari* 234^T; 6, *F. placidus* AEDII12DO^T. 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.

Characteristic	1	2	3	4	5	6
Habitat	Deep-sea hydrothermal system, Suiyo Seamount	Hot sediment of hydrothermal system, Vulcano, Italy	Deep-sea hydrothermal system, Guaymas, Mexico	Deep-sea wall of black smoker, Mid-Atlantic Ridge	Deep-sea hydrothermal system, Guaymas, Mexico	Sand/water of shallow beach, Vulcano, Italy
S ⁰ sensitivity	Inhibitory	Inhibitory	Inhibitory	Inhibitory	ND	Inhibitory
CH ₄ formation	+	+	–	+	–	–
Fluorescence under UV	+	+	+	+	–	–
Chemolithoautotrophic growth	–	H ₂ /CO ₂ + S ₂ O ₃ ^{2–}	–	H ₂ /CO ₂ + S ₂ O ₃ ^{2–} ; H ₂ /CO ₂ + S ₂ O ₃ ^{2–}	H ₂ /CO ₂ + Fe ³⁺	H ₂ /CO ₂ + S ₂ O ₃ ^{2–} ; H ₂ /CO ₂ + NO ₃ [–] ; FeS/CO ₂ + NO ₃ [–]
Growth requirements	Acetate, coenzyme M	–	Organic carbon sources	–	–	–
Optimum growth conditions:						
Temperature (°C)	70	83	82	80	88	85
pH	6.5	(5.5–7.5)*	6.0	7.0	7.0	7.0
Use of electron acceptors:						
SO ₄ ^{2–}	–	+	+	–	–	–
SO ₃ ^{2–}	+	+	+	+	–	–
S ₂ O ₃ ^{2–}	+	+	+	+	–	+
S ⁰	–	–	–	ND	–	–
NO ₃ [–]	–	–†	–†	–	–	+
NO ₂ [–]	–	–†	–†	–	–	–
Fe ³⁺	–	–	–	–	+	+
DNA G + C content (HPLC; mol%)	45.9	47.6†	43.7†	46.2†	58.7	43
DNA–DNA hybridization (%) with:						
Strain Arc51 ^T	100	4	1	16	ND	ND
<i>A. veneficus</i> SNP6 ^T	17	5	4	100	ND	ND

*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. veneficus* could grow at 80 °C, but the isolate was intolerant of this high temperature. On the basis of the data described above, therefore, strain Arc51^T represents a novel species within the genus *Archaeoglobus*, for which the name *Archaeoglobus infectus* sp. nov. is proposed.

An interesting feature of strain Arc51^T 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. placidus* (Vorholt *et al.*, 1997), although whether the trace methanogenesis is fundamentally connected to their growth is open to investigation. Strain Arc51^T 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 Arc51^T, 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 sulfite 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, Arc51^T (=NBRC 100649^T=DSM 18877^T), was isolated from a rock sample collected in a hydrothermal vent in Suiyo Seamount, Izu-Bonin Arc, western Pacific Ocean.

Acknowledgements

We thank the team that operated the benthic multi-coring system and the crew of the *Dainihakureimaru* for collecting the samples. We thank Ms Kuniko Shimamura for technical support. The Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan funded part of this research through the Special Coordination Fund ('Archaeon Park Project': International Research Project on Interaction Between SubVent Biosphere and Geo-Environments).

References

- Achenbach, L. & Woese, C. (1995). 16S and 23S rRNA-like primers. In *Archaea: a Laboratory Manual*, pp. 201–203. Edited by F. T. Robb, A. R. Place, K. R. Sowers, H. J. Schreier, S. DasSarma & E. M. Fleischmann. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Allen, J. R., Clark, D. D., Krum, J. G. & Ensign, S. A. (1999). A role for coenzyme M (2-mercaptoethanesulfonic acid) in a bacterial pathway of aliphatic epoxide carboxylation. *Proc Natl Acad Sci U S A* **96**, 8432–8437.
- Boyd, J. M., Ellsworth, A. & Ensign, S. A. (2006). Characterization of 2-bromoethanesulfonate as a selective inhibitor of the coenzyme M-dependent pathway and enzymes of bacterial aliphatic epoxide metabolism. *J Bacteriol* **188**, 8062–8069.
- Burggraf, S., Jannasch, H. W., Nicolaus, B. & Stetter, K. O. (1990). *Archaeoglobus profundus* sp. nov., represents a new species within the sulfate-reducing *Archaeobacteria*. *Syst Appl Microbiol* **13**, 24–28.
- Coleman, N. V. & Spain, J. C. (2003a). Epoxyalkane: coenzyme M transferase in the ethene and vinyl chloride biodegradation pathways of *Mycobacterium* strain JS60. *J Bacteriol* **185**, 5536–5545.
- Coleman, N. V. & Spain, J. C. (2003b). Distribution of the coenzyme M pathway of epoxide metabolism among ethene- and vinyl chloride-degrading *Mycobacterium* strains. *Appl Environ Microbiol* **69**, 6041–6046.
- Danko, A. S., Saski, C. A., Tomkins, J. P. & Freedman, D. L. (2006). Involvement of coenzyme M during aerobic biodegradation of vinyl chloride and ethene by *Pseudomonas putida* strain AJ and *Ochrobactrum* sp. strain TD. *Appl Environ Microbiol* **72**, 3756–3758.
- Friedrich, M. W. (2002). Phylogenetic analysis reveals multiple lateral transfers of adenosine-5'-phosphosulfate reductase genes among sulfate-reducing microorganisms. *J Bacteriol* **184**, 278–289.
- Glasby, G. P., Iizasa, K., Yuasa, M. & Usui, A. (2000). Submarine hydrothermal mineralization on the Izu-Bonin Arc, south of Japan: an overview. *Mar Georesour Geotechnol* **18**, 141–176.
- Graham, D. E. & White, R. H. (2002). Elucidation of methanogenic coenzyme biosyntheses: from spectroscopy to genomics. *Nat Prod Rep* **19**, 133–147.
- Hafenbradl, D., Keller, M., Dirmeier, R., Rachel, R., Rossnagel, P., Burggraf, S., Huber, H. & Stetter, K. O. (1996). *Ferroglobus placidus* gen. nov., sp. nov., a novel hyperthermophilic archaeum that oxidizes Fe²⁺ at neutral pH under anoxic conditions. *Arch Microbiol* **166**, 308–314.

- Hara, K., Kakegawa, T., Yamashiro, K., Maruyama, A., Ishibashi, J., Marumo, K., Urabe, T. & Yamagishi, A. (2005). Analysis of the archaeal sub-seafloor community at Suiyo Seamount on the Izu-Bonin Arc. *Adv Space Res* 35, 1634–1642.
- Hattori, S., Kamagata, Y., Hanada, S. & Shoun, H. (2000). *Thermacetogenium phaeum* gen. nov., sp. nov., a strictly anaerobic, thermophilic, syntrophic acetate-oxidizing bacterium. *Int J Syst Evol Microbiol* 50, 1601–1609.
- Higashi, Y., Sunamura, M., Kitamura, K., Nakamura, K.-I., Kurusu, Y., Ishibashi, J.-I., Urabe, T. & Maruyama, A. (2004). Microbial diversity in hydrothermal surface to subsurface environments of Suiyo Seamount, Izu-Bonin Arc, using a catheter-type in situ growth chamber. *FEMS Microbiol Ecol* 47, 327–336.
- Huber, H., Jannasch, H., Rachel, R., Fuchs, T. & Stetter, K. O. (1997). *Archaeoglobus veneficus* sp. nov., a novel facultative chemolithoautotrophic hyperthermophilic sulfite reducer, isolated from abyssal black smokers. *Syst Appl Microbiol* 20, 374–380.
- Itoh, T., Suzuki, K., Sanchez, P. C. & Nakase, T. (1999). *Caldivirga maquilingensis* gen. nov., sp. nov., a new genus of rod-shaped crenarchaeote isolated from a hot spring in the Philippines. *Int J Syst Bacteriol* 49, 1157–1163.
- Kashefi, K., Tor, J. M., Holmes, D. E., Gaw Van Praagh, C. V., Reysenbach, A. L. & Lovley, D. R. (2002). *Geoglobus ahangari* gen. nov., sp. nov., a novel hyperthermophilic archaeon capable of oxidizing organic acids and growing autotrophically on hydrogen with Fe(III) serving as the sole electron acceptor. *Int J Syst Evol Microbiol* 52, 719–728.
- Klenk, H. P., Clayton, R. A., Tomb, J. F., White, O., Nelson, K. E., Ketchum, K. A., Dodson, R. J., Gwinn, M., Hickey, E. K. & other authors (1997). The complete genome sequence of the hyperthermophilic, sulphate-reducing archaeon *Archaeoglobus fulgidus*. *Nature* 390, 364–370.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* 32, 1363–1371.
- Mattes, T. E., Coleman, N. V., Spain, J. C. & Gossett, J. M. (2005). Physiological and molecular genetic analyses of vinyl chloride and ethene biodegradation in *Nocardioides* sp. strain JS614. *Arch Microbiol* 183, 95–106.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 39, 159–167.
- Miller, T. L., Wolin, M. J., Zhao, H. X. & Bryant, M. P. (1986). Characteristics of methanogens isolated from bovine rumen. *Appl Environ Microbiol* 51, 201–202.
- Mori, K., Yamamoto, H., Kamagata, Y., Hatsu, M. & Takamizawa, K. (2000). *Methanocalculus pumilus* sp. nov., a heavy-metal-tolerant methanogen isolated from a waste-disposal site. *Int J Syst Evol Microbiol* 50, 1723–1729.
- NBRC (2005). *NBRC Catalogue of Biological Resources: Microorganisms, Genomic DNA Clones, and cDNAs*. Chiba, Japan: National Institute of Technology and Evaluation (NITE).
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4, 406–425.
- Sprenger, W. W., van Belzen, M. C., Rosenberg, J., Hackstein, J. H. & Keltjens, J. T. (2000). *Methanomicrococcus blatticola* gen. nov., sp. nov., a methanol- and methylamine-reducing methanogen from the hindgut of the cockroach *Periplaneta americana*. *Int J Syst Evol Microbiol* 50, 1989–1999.
- Stetter, K. O. (1988). *Archaeoglobus fulgidus* gen. nov., sp. nov. a new taxon of extremely thermophilic Archaeobacteria. *Syst Appl Microbiol* 10, 172–173.
- Stetter, K. O., Lauerer, G., Thomm, M. & Neuner, A. (1987). Isolation of extremely thermophilic sulfate reducers: evidence for a novel branch of archaeobacteria. *Science* 236, 822–824.
- Stetter, K. O., Huber, R., Blöchl, E., Kurr, M., Eden, R. D., Feilder, M., Cash, H. & Vance, I. (1993). Hyperthermophilic archaea are thriving in deep North Sea and Alaskan oil reservoirs. *Nature* 365, 743–745.
- Tamaki, H., Hanada, S., Kamagata, Y., Nakamura, K., Nomura, N., Nakano, K. & Matsumura, M. (2003). *Flavobacterium limicola* sp. nov., a psychrophilic, organic-polymer-degrading bacterium isolated from freshwater sediments. *Int J Syst Evol Microbiol* 53, 519–526.
- Taylor, C. D. & Wolfe, R. S. (1974). Structure and methylation of coenzyme M (HSC₂CH₂SO₃). *J Biol Chem* 249, 4879–4885.
- Taylor, C. D., McBride, B. C., Wolfe, R. S. & Bryant, M. P. (1974). Coenzyme M, essential for growth of a rumen strain of *Methanobacterium ruminantium*. *J Bacteriol* 120, 974–975.
- Thauer, R. K. (1998). Biochemistry of methanogenesis: a tribute to Marjory Stephenson. 1998 Marjory Stephenson Prize Lecture. *Microbiology* 144, 2377–2406.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 25, 4876–4882.
- Tor, J. M., Kashefi, K. & Lovley, D. R. (2001). Acetate oxidation coupled to Fe(III) reduction in hyperthermophilic microorganisms. *Appl Environ Microbiol* 67, 1363–1365.
- Tsunogai, U., Ishibashi, J., Wakita, H., Gamo, T., Watanabe, K., Kajimura, T., Kanayama, S. & Sakai, H. (1994). Peculiar features of Suiyo Seamount hydrothermal fluids, Izu-Bonin Arc: differences from subaerial volcanism. *Earth Planet Sci Lett* 126, 289–301.
- Vargas, M., Kashefi, K., Blunt-Harris, E. L. & Lovley, D. R. (1998). Microbiological evidence for Fe(III) reduction on early Earth. *Nature* 395, 65–67.
- Vorholt, J., Kunow, J., Stetter, K. O. & Thauer, R. K. (1995). Enzymes and coenzymes of the carbon monoxide dehydrogenase pathway for autotrophic CO₂ fixation in *Archaeoglobus lithotrophicus* and the lack of carbon monoxide dehydrogenase in the heterotrophic *A. profundus*. *Arch Microbiol* 163, 112–118.
- Vorholt, J. A., Hafenbradl, D., Stetter, K. O. & Thauer, R. K. (1997). Pathways of autotrophic CO₂ fixation and of dissimilatory nitrate reduction to N₂O in *Ferroglobus placidus*. *Arch Microbiol* 167, 19–23.
- Wagner, M., Roger, A. J., Flax, J. L., Brusseau, G. A. & Stahl, D. A. (1998). Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. *J Bacteriol* 180, 2975–2982.