**Geoglobus acetivorans** sp. nov., an iron(III)-reducing archaeon from a deep-sea hydrothermal vent

G. B. Slobodkina,¹ T. V. Kolganova,² J. Querellou,³ E. A. Bonch-Osmolovskaya¹ and A. I. Slobodkin¹

Correspondence
G. B. Slobodkina
gslobodkina@mail.ru

¹Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, 117312 Moscow, Russia
²Bioengineering Center, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/1, 117312 Moscow, Russia
³UMR 6197, Microbiology of Extreme Environments, Ifremer, Centre de Brest, 29280 Plouzané, France

A hyperthermophilic, anaerobic, dissimilatory Fe(III)-reducing, facultatively chemolithoautotrophic archaeon (strain SBH6T) was isolated from a hydrothermal sample collected from the deepest of the known World Ocean hydrothermal fields, Ashadze field (12° 58’ 21” N 44° 51’ 47” W) on the Mid-Atlantic Ridge, at a depth of 4100 m. The strain was enriched using acetate as the electron donor and Fe(III) oxide as the electron acceptor. Cells of strain SBH6T were irregular cocci, 0.3–0.5 μm in diameter. The temperature range for growth was 50–85 °C, with an optimum at 81 °C. The pH range for growth was 5.0–7.5, with an optimum at pH 6.8. Growth of SBH6T was observed at NaCl concentrations ranging from 1 to 6 % (w/v) with an optimum at 2.5 % (w/v). The isolate utilized acetate, formate, pyruvate, fumarate, malate, propionate, butyrate, succinate, glycerol, stearate, palmitate, peptone and yeast extract as electron donors for Fe(III) reduction. It was also capable of growth with H2 as the sole electron donor, CO2 as a carbon source and Fe(III) as an electron acceptor without the need for organic substances. Fe(III) in the form of poorly crystalline Fe(III) oxide or Fe(III) citrate was the only electron acceptor that supported growth. 16S rRNA gene sequence analysis revealed that the closest relative of the isolated organism was *Geoglobus ahangari* sp. nov. is proposed. The type strain is SBH6T (=DSM 21716T =VKM B-2522T).

Iron minerals are abundant in deep-sea hydrothermal vents. The surfaces of active chimneys are frequently covered with deposits of iron oxides in different oxidative states, and the amount of iron in hydrothermal fluid can reach molar concentrations. Thus, deep-sea hydrothermal vents can provide an ecological niche for Fe(III)-reducing micro-organisms (Slobodkin et al., 2001). However, only a few thermophilic Fe(III)-reducers have been isolated from this environment. Currently, thermophilic and hyperthermophilic iron-reducing micro-organisms recovered from deep-sea habitats include two species of the *Bacteria*, *Geothermobacter ehrlichii* (Kashefi et al., 2003) and *Deferribacter abyssi* (Miroshnichenko et al., 2003), and three representatives of the *Archaea*, *Thermococcus* sp. SN531 (Slobodkin et al., 2001), *Geoglobus ahangari* (Kashefi et al., 2002) and 'Candidatus Aciduliprofundum boonei' (Reysenbach et al., 2006). In this paper, we report the isolation and characterization of a novel hyperthermophilic Fe(III)-reducing archaeon from the deepest of the known World Ocean hydrothermal fields.

Strain SBH6T was isolated from a fragment of a hydrothermal chimney-like structure. The sample was collected in March 2007 during the Serpentine cruise at the Ashadze hydrothermal field (12° 58’ 21” N 44° 51’ 47” W) on the Mid-Atlantic Ridge at a depth of 4100 m. For sample collection, sterilized microbiological boxes filled with sterile freshwater were prepared onboard. Active chimney samples were collected by the ROV Victor. On site, after opening the box lid, the freshwater was replaced.

**Abbreviations:** AQDS, 9,10-anthraquinone-2,6-disulfonate; DGGE, denaturing gradient gel electrophoresis.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SBH6T is FJ216404.
by *in situ* seawater, the chimney fragment was introduced and the lid was closed. The following operations were done onboard under sterile conditions. Boxes with samples were stored at 4 °C. An enrichment culture was initiated by inoculation of 10 % (v/v) of the sample into anaerobically prepared, bicarbonate-buffered, sterile (135 °C, 1 h) liquid medium with acetate (18 mM) as an electron donor and poorly crystalline Fe(III) oxide (90 mmol l⁻¹) as an electron acceptor. Medium composition and preparation techniques were as described previously (Slobodkin et al., 1999a); the medium was additionally supplemented with NaCl (18 g l⁻¹) and MgCl₂ (4 g l⁻¹) to increase the salinity. After three subsequent transfers and following serial 10-fold dilutions in the same medium at 65 °C, two morphological cell-types were observed in the highest positive dilution (10⁻⁵): rods and coccii. To obtain coccoid-shaped micro-organisms, further cultivation was carried out at 82 °C. After two transfers at 82 °C, rods were not observed at either 82 or 65 °C and only coccoid-shaped cells were present in the enrichment. Acetate (4.5 mM) consumption in enrichments, measured by gas chromatography, reached 80 %, Attempts to obtain separate colonies in agar-blocks or by the roll-tube method (Hungate, 1969) were unsuccessful at either 82 or 65 °C with 1 % Gelrite gellan gum or 2 % agar as the solidifying agent in the medium, respectively. The enrichment was subsequently transferred five times under lithoautotrophic conditions with molecular hydrogen as an electron donor, poorly crystalline Fe(III) oxide as an electron acceptor and CO₂ as the carbon source at 82 °C and, after that, serially diluted under the same conditions. The isolate from the highest dilution that exhibited Fe(III) reduction (10⁻⁶) was considered as a pure culture and was designated strain SBH6T. Denaturing gradient gel electrophoresis (DGGE) of strain SBH6T grown on medium containing acetate (18 mM) or peptone (10 g l⁻¹) as electron donor and poorly crystalline Fe(III) oxide (90 mmol l⁻¹) as electron acceptor in the presence of yeast extract (0.02 g l⁻¹) revealed a single band in each case and thus confirmed the purity of the culture. Complete 16S rRNA gene sequences of strain SBH6T grown with acetate or peptone were identical. Physiological studies on substrate and electron acceptor utilization, temperature, pH and salinity ranges for growth, light and electron microscopy, analytical techniques [Fe(II) and acetate concentrations] and DNA extraction were performed as described previously (Slobodkin et al., 1999a). Growth of the strain with poorly crystalline Fe(III) was determined by direct cell-count using light microscopy after dissolving the iron precipitate in a solution of ammonium oxalate (28 g l⁻¹)/oxalic acid (15 g l⁻¹) (Lovley & Phillips, 1988). pH measurements and pH-meter calibration were carried out at 60 °C. 16S rRNA gene amplification, sequencing and sequence analysis were done as described previously (Zavarzina et al., 2002). 16S rRNA gene fragments for DGGE were obtained using PCR with primers Uni515F (Lane, 1991) with GC-clamp (Muyzer et al., 1993) at the 5’ end and 915R (Casamayor et al., 2002). DGGE was performed as described by Muyzer et al., (1997) with a denaturing gradient ranging from 35 to 65 % (100 % denaturant contains 7 M urea and 40 % formamide).

Cells of strain SBH6T were regular to irregular coccii, approximately 0.3–0.5 μm in diameter, usually arranged as single cells; flagella were not observed. The temperature range for growth of strain SBH6T was 50–85 °C, with an optimum at 81 °C. No growth was detected at 90 or 46 °C after incubation for 3 weeks. The pH range for growth was 5.0–7.5, with an optimum at pH 6.8. No growth was observed at pH 4.5 or 8.0. Growth of strain SBH6T was observed at NaCl concentrations ranging from 1.0 to 6.0 % (w/v) with an optimum at 2.5 % (w/v), but no growth was evident at 0 or 7 % NaCl (w/v). Poorly crystalline Fe(III) oxide was reduced to a black magnetic precipitate with high Fe(II) content. No changes in colour or amount of precipitate were observed in uninoculated controls containing poorly crystalline Fe(III) oxide during the incubation period at 82 °C. Strain SBH6T grew and reduced Fe(III) with acetate (4.5 or 18 mM), formate, pyruvate, fumarate, malate, propionate, butyrate, succinate, glycerol (20 mM each), stearate, palmitate (0.5–1.0 mM each), peptone or yeast extract (10 g l⁻¹ each). During growth with acetate (4.5 mM) and Fe(III) in the absence of yeast extract, 80 % of the acetate was consumed and the ratio of Fe(II) produced to acetate consumed was 7.5. Strain SBH6T also grew with molecular hydrogen as the sole electron donor for Fe(III) reduction and CO₂ as the carbon source. No organic carbon source was required for growth on hydrogen. There was no Fe(III) reduction or cell growth in the absence of added hydrogen. Strain SBH6T was not able to utilize lactate (25 mM), l-alanine, glycine (20 mM each), l-proline, arginine, serine, glutamate, asparagine, l-cysteine, aspartic acid, glutamic acid (10 mM each), methanol, ethanol or benzoate (20 mM each) with poorly crystalline Fe(III) oxide as an electron acceptor. Besides poorly crystalline Fe(III) oxide, strain SBH6T could also grow with Fe(III) citrate (10 mM) as the electron acceptor, but cell-yield and Fe(III) reduction were lower. Fe(III) citrate was not completely reduced; no more than 5–6 mM of Fe(II) was formed. Several attempts to grow strain SBH6T on a variety of commonly considered electron acceptors [including sulfate (14 mM), thiosulfate (20 mM), l-methionine (10 g l⁻¹), nitrate (10 mM), fumarate (20 mM), Mn(IV) oxide (25 mM), 9,10-anthraquinone-2,6-disulfonate (AQDS; 20 mM) and oxygen (2 or 20 %, v/v)] other than poorly crystalline Fe(III) oxide (90 mmol l⁻¹) and Fe(III) citrate (10 mM) using acetate (18 mM), H₂ (as H₂/CO₂, 80/20, v/v, 101 kPa), lactate (25 mM), butyrate (20 mM) or glycerol (20 mM) were unsuccessful.

A comparison of 1417 nucleotides of the 16S rRNA gene sequence of strain SBH6T with those available in the GenBank database showed that strain SBH6T had the highest identity with *G. ahangari* 234T (97 %) (Fig. 1). Only 16S rRNA gene sequences of the type strains of species with validly published names were included in the analyses. Levels of 16S rRNA gene sequence similarity with...
other members of the order *Archaeoglobales* were 94.9–95.3%. Trees constructed by using maximum-likelihood and maximum-parsimony algorithms displayed the same topology (data not shown). Transversion analysis (Woese et al., 1991) did not affect the phylogenetic position of the novel strain.

![Phylogenetic tree](image)

The new hyperthermophilic isolate described in this report, capable of reduction of Fe(III), was recovered from an environmental sample by using acetate as the electron donor for initial enrichment. Acetate is one of the major metabolic products of organic matter decomposition under anaerobic conditions and it could be produced during fermentation by many hyperthermophiles (Slobodkin et al., 1999b). For a long time, there were no data demonstrating anaerobic acetate degradation by hyperthermophiles. Recently, this ability was shown for two micro-organisms, *Ferroglobus placidus* and *G. ahangari* (Tor et al., 2001); however, none of the strains of either of these species were initially enriched and obtained in pure culture with acetate as the electron donor. Final purification of isolate SBH6\(^T\) was carried out under lithoautotrophic conditions with molecular hydrogen as the electron donor, since colonies were not formed on medium with acetate. However, only one morphological cell-type was observed in enrichments cultivated under hyperthermophilic conditions. Since the same extent of acetate consumption and Fe(III) reduction was detected before and after purification under autotrophic conditions, we can assume that strain SBH6\(^T\)\) was responsible for acetate utilization in initial enrichments. Therefore, SBH6\(^T\) is the first hyperthermophilic micro-organism enriched on acetate as the electron donor. At present, the order *Archaeoglobales* includes one family, *Archaeoglobaceae*, consisting of three genera: *Archaeoglobus*, *Ferroglobus* and *Geoglobus* (Cole et al., 2007). The genus *Geoglobus* is represented by the sole species, *G. ahangari* (Kashefi et al., 2002) isolated from a deep-sea hydrothermal sample from Guaymas Basin, Gulf of California. Isolate SBH6\(^T\) shared many phenotypic features with the described representative of this genus. Firstly, they are unable to utilize electron acceptors other than Fe(III), preferably insoluble Fe(III) oxide, for growth. Both micro-organisms grew poorly in media with soluble forms of Fe(III) [Fe(III) citrate] as an electron acceptor. There was practically no difference in electron donor utilization, including anaerobic oxidation of long-chain fatty acids and the ability to grow chemolithoautotrophically. Significant differences were observed in growth temperature between *G. ahangari* and strain SBH6\(^T\). Strain SBH6\(^T\) grew at 50 °C and did not grow at 85 °C and above, whereas the low growth limit for *G. ahangari* was 65 °C and it was able to grow at up to 90 °C. The optimal growth temperatures also differed by 7 °C, being 88 °C for *G. ahangari* and 81 °C for strain SBH6\(^T\). In addition, analysis of 16S rRNA gene sequences revealed considerable phylogenetic distance between *G. ahangari* and isolate SBH6\(^T\). Thus, phylogenetic and physiological properties clearly differentiate strain SBH6\(^T\) from the closest relative, *G. ahangari*. According to the opinion of the Judicial Commission of the International Committee for Systematics of Prokaryotes, (2008), the name *G. ahangari* is not validly published since the type strain for this species is deposited only in one collection of micro-organisms. However, we hope that the type strain will be deposited in a second collection so that this name will be validly published and we propose strain SBH6\(^T\) as the type strain of the novel species, *Geoglobus acitivorans* sp. nov., the second species of the genus *Geoglobus*.

**Description of Geoglobus acitivorans** sp. nov.

*Geoglobus acitivorans* (a.ca.ti.vo’rans. L. neut. n. acetum vinegar, used to refer to acetic acid; L. part. adj. vorans devouring; N.L. part. adj. acitivorans vinegar consuming). Cells are regular to irregular cocci, 0.3–0.5 μm in diameter, occurring singly. The temperature range for growth is 50–85 °C, with an optimum at 81 °C. The pH range for growth is 5.0–7.5, with an optimum at pH 6.8. Growth occurs at NaCl concentrations ranging from 1 to 6 % (w/v) with an optimum at 2.5 % (w/v). Anaerobic. Capable of chemolithoautotrophic growth using molecular hydrogen as an electron donor, ferric iron as electron acceptor and CO2 as the carbon source. Only poorly crystalline Fe(III) oxide and Fe(III) citrate are used as electron acceptors for growth. Sulfate, thiosulfate, elemental sulfur, nitrate, fumarate, Mn(IV) oxide, AQDS and oxygen are not utilized as electron acceptors. With poorly crystalline Fe(III) oxide, anaerobically oxidizes acetate, formate, pyruvate, fumarate, malate, propionate, butyrate, succinate, glycerol, stearate, palmitate, peptone and yeast extract. Lactate, L-alanine,
glycine, L-proline, arginine, serine, glutamine, asparagine, L-cysteine, aspartic acid, glutamic acid, methanol, ethanol and benzoate are not utilized.

The type strain is SBH6T (=DSM 21716T =VKM B-2522T), isolated from a deep-sea hydrothermal field (Ashadze) of the Mid-Atlantic Ridge.

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References


