Desulfurococcus fermentans sp. nov., a novel hyperthermophilic archaeon from a Kamchatka hot spring, and emended description of the genus Desulfurococcus

A. A. Perevalova,² V. A. Svetlichny,³ I. V. Kublanov,¹ N. A. Chernyh,¹ N. A. Kostrikina,¹ T. P. Tourova,¹ B. B. Kuznetsov³ and E. A. Bonch-Osmolovskaya¹

¹Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, Moscow 117312, Russia
²Lehrstuhl für Mikrobiologie, Universität Bayreuth, D-95440 Bayreuth, Germany
³Bioengineering Center, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/1, Moscow 117312, Russia

An obligately anaerobic, hyperthermophilic, organoheterotrophic archaeon, strain Z-1312 T, was isolated from a freshwater hot spring of the Uzon caldera (Kamchatka Peninsula, Russia). The cells were regular cocci, 1–4 μm in diameter, with one long flagellum. The cell envelope was composed of a globular layer attached to the cytoplasmic membrane. The temperature range for growth was 63–89 °C, with an optimum between 80 and 82 °C. The pH range for growth at 80 °C was 4.8–6.8, with an optimum at pH 6.0. Strain Z-1312T grew by hydrolysis and/or fermentation of a wide range of polymeric and monomeric substrates, including agarose, amygdalin, arabinose, arbutin, casein hydrolysate, cellulose (filter paper, microcrystalline cellulose, carboxymethyl cellulose), dextran, dulcitol, fructose, lactose, laminarin, lichenan, maltose, pectin, peptone, ribose, starch and sucrose. No growth was detected on glucose, xylose, mannitol or sorbitol. Growth products when sucrose or starch were used as the substrate were acetate, H₂ and CO₂. Elemental sulfur, thiosulfate and nitrate added as potential electron acceptors for anaerobic respiration did not stimulate growth when tested with starch as the substrate. H₂ at 100 % in the gas phase did not inhibit growth on starch or peptone. The G+C content of the DNA was 42.5 mol%. 16S rRNA gene sequence analysis placed the isolated strain Z-1312T as a member of the genus Desulfurococcus, where it represented a novel species, for which the name Desulfurococcus fermentans sp. nov. (type strain Z-1312T = DSM 16532 T = VKM V-2316 T) is proposed.

Anaerobic, hyperthermophilic prokaryotes with fermentative metabolism have been identified in both archaeal kingdoms, Euryarchaeota and Crenarchaeota (Blochl et al., 1995; Stetter, 1996; Huber et al., 2000). Usually, these organisms grow on complex media with proteins or peptides as the energy substrates, producing volatile fatty acids, CO₂ and H₂ as fermentation products. Molecular hydrogen often inhibits growth of fermentative archaea; elimination of H₂ by reduction of elemental sulfur to H₂S, by flushing the cultures with an inert gas (Fiala & Stetter, 1986) or by cultivation in co-cultures with H₂-consuming methanogens (Bonch-Osmolovskaya & Stetter, 1991) supports the growth of fermentative archaea. Thus, growth of most fermentative archaea in closed vessels is either obligately dependent on elemental sulfur or stimulated by its presence. An exception is the fermentation observed with two representatives of the Crenarchaeota, Sulfothermoarculocococcus zilligii (Hensel et al., 1997) and Thermoproteus aggregans (Huber et al., 1998), which do not require elemental sulfur and are inhibited by its presence. All members of the genus Desulfurococcus reported to date (Zillig et al., 1982; Bonch-Osmolovskaya et al., 1988) grow exclusively or preferably on peptides, concomitantly reducing elemental sulfur to H₂S. Their growth on peptides is stimulated significantly by the presence of elemental sulfur and inhibited by H₂ (Slobodkin & Bonch-Osmolovskaya, 1994). Desulfurococcus amylolyticus (Bonch-Osmolovskaya et al., 1988; Tourova

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Desulfurococcus fermentans Z-1312 is AY264344.
et al., 2000) can grow on starch, glycogen or pectin, but its growth on these substrates is much weaker in comparison with that on peptides and also depends on the presence of S0. Here, we describe a strain representing a novel hyperthermophilic species of the genus *Desulfurococcus*, *Desulfurococcus fermentans*, isolated from a Kamchatka hot spring. The organism grows by fermentation of diverse carbohydrates, including cellulose, does not require the presence of elemental sulfur and is not inhibited by H2. To our knowledge, this is the first report on an archaeon that is able to grow on cellulosic substrates.

Samples of water and mud from a freshwater hot spring of the Uzon caldera (Kamchatka Peninsula, Russia) were used for inoculation of anaerobically prepared basal medium of the following composition (mg l⁻¹ unless indicated): KCl, 330; NH₄Cl, 330; KH₂PO₄, 330; MgCl₂.6H₂O, 330; CaCl₂.2H₂O, 330; Na₂S.9H₂O, 500; starch, 5000; yeast extract (Difco), 200; resazurin, 1; trace element solution (Pfennig & Lippert, 1965), 1 ml l⁻¹; vitamin solution (Wolin et al., 1963), 1 ml l⁻¹; pH 6.5 (adjusted with H₂SO₄). The medium was prepared anaerobically under an atmosphere of 80 % N₂ + 20 % CO₂ and dispensed into 15 ml Hungate tubes with butyl rubber stoppers, leaving 5 ml as the headspace.

After 3–5 days incubation at 85 °C, an organism with regular coccoid cells, often forming sarcina-like aggregates, was enriched from the Uzon caldera sample. In pure culture obtained by serial dilutions of the initial enrichment, the cells were present only as single coccoid cells. The new isolate was designated strain Z-1312T.

Cells of strain Z-1312T were regular cocci, 1–4 μm in diameter (Fig. 1a). On electron micrographs of whole cells, a long single flagellum is present. Thin sections (Bonch-Osmolovskaya et al., 1990) revealed a cell-wall structure consisting of a cellular membrane covered by one layer of subunits (Fig. 1b). Strain Z-1312T was an obligate anaerobe, since no growth was observed under oxic conditions as well as under anoxic conditions when the medium was not pre-reduced by the addition of sodium sulfide. The organism was a hyperthermophile, growing in the temperature range 63–89 °C with an optimum at 80–82 °C. Strain Z-1312T grew over a pH range of 4.8–6.8, with an optimum at pH 6.0.

Strain Z-1312T grew by fermentation of starch (5 g l⁻¹) or cellulose (2 g l⁻¹) (filter paper, microcrystalline cellulose, carboxymethyl cellulose) as well as of a wide range of monomeric and polymeric substrates (2 g l⁻¹), including agarose, arabinose, arbutin, casein hydrolysate, dulcitol, pectin, peptone, sucrose and yeast extract, with a final cell yield of 2.5–5.0 × 10⁷ cells ml⁻¹. Weaker growth resulting in a cell yield around or below 2.0 × 10⁶ cells ml⁻¹ was observed on amygdalin, dextran, fructose, lactose, laminarin, lichenan, maltose and ribose. No growth was detected on glucose, xylose, mannitol or sorbitol. Growth curves of strain Z-1312T on media with starch and microcrystalline cellulose are shown in Fig. 2. The final cell yield on starch

![Fig. 1.](image1.png)

**Fig. 1.** Electron micrographs of cells of strain Z-1312T. (a) Negatively stained whole cell showing a single flagellum. (b) Thin section. Bars, 0.5 μm.

![Fig. 2.](image2.png)

**Fig. 2.** Growth of strain Z-1312T on media containing 0.2 g yeast extract l⁻¹ alone (△) or additionally supplemented with 5 g starch l⁻¹ (○) or 5 g microcrystalline cellulose l⁻¹ (◇). The incubation temperature was 82 °C and the pH of the media was 6.0.
was $5 \times 10^7$ cells ml$^{-1}$, while the doubling time was 6·3 h. On microcrystalline cellulose, the final cell yield was around $2\cdot5 \times 10^7$ cells ml$^{-1}$ and the doubling time was 10·7 h. The only growth products (Bonch-Osmolovskaya & Miroshnichenko, 1994) detected during growth on media with starch or sucrose were acetate, H$_2$ and CO$_2$. Growth on cellulosic substrates, peptone, starch and sucrose was found to be stimulated by yeast extract (optimum concentration 200 mg l$^{-1}$). The ability of strain Z-1312$^T$ to use elemental sulfur as an electron acceptor was tested in cultures growing on starch and peptone. Although sulfur was reduced to hydrogen sulfide, no effect of the presence of sulfur on the cell yield was observed. Thiosulfate, sulfate and nitrate were not reduced and did not influence growth on starch or peptone. H$_2$ (100 % in the gas phase) did not inhibit growth on starch and peptone. Although sulfur was reduced to hydrogen sulfide, no effect of the presence of sulfur on the cell yield was observed. Thiosulfate, sulfate and nitrate were not reduced and did not influence growth on starch or peptone. H$_2$ (100 % in the gas phase) did not inhibit growth on starch and peptone.

The G+C content of the DNA of strain Z-1312$^T$ was determined by the denaturation method (Owen & Lapage, 1976) as 42·5 mol%. Analysis of partial 16S rRNA gene sequences of strain Z-1312$^T$ (1378 nucleotides) was done as described previously (Sanger et al., 1977) using $5'$-AGAGTTTGATCCTGCTCAG-3' as the forward primer and $5'$-TACGGTTACCTTGTTACGACTT-3' as the reverse primer (Lane, 1991). This sequence has a high G+C content (65-0 %), as observed previously for the 16S rRNA genes of other thermophilic prokaryotic organisms. Primary analysis of 16S rRNA gene nucleotide sequence similarity of the new isolate was carried out using the BLASTA server (http://www.ncbi.nlm.nih.gov/blast). The sequences were aligned against the corresponding 16S rRNA gene sequences of related organisms using the CLUSTAL program (Thompson et al., 1994). Positions that had not been sequenced in one or more reference organisms were omitted and a total of 1193 nucleotides were used in the analysis. The phylogenetic tree rooted by the outgroup Methanococcus vannielii was constructed by the neighbour-joining method with bootstrap analysis of 100 trees using the programs of the TREECON package (Van de Peer & De Wachter, 1994). This analysis revealed that strain Z-1312$^T$ was a member of the family Desulfurococaceae (Burggraf et al., 1997) (kingdom Crenarchaeota, domain Archaea). Additional sequence alignments and phylogenetic analysis performed with members of this family revealed that strain Z-1312$^T$ was closely related to the species of the genus Desulfurococcus (95-5–96-4 % sequence similarity). In the phylogenetic tree (Fig. 3), strain Z-1312$^T$ formed a single cluster with Desulfurococcus species with a high level of bootstrap probability of this branching point (96 %).

The genus Desulfurococcus (Zillig et al., 1982) at present consists of three species with validly published names: Desulfurococcus mucosus, the type species of the genus, Desulfurococcus mobilis (Zillig et al., 1982) and D. amylolyticus (Bonch-Osmolovskaya et al., 1988; Tourova et al., 2000). Nucleotide sequences of the 16S rRNA gene have been obtained only for the last two species. Representatives of these species could be detected by specific oligonucleotide probes (Perevalova et al., 2003). The affiliation of isolate Z-1312$^T$ to the genus Desulfurococcus is in good agreement with common phenotypic features that exist between strain Z-1312$^T$ and other representatives of the genus Desulfurococcus (Table 1). However, although it shows the same cell morphology and growth characteristics, strain Z-1312$^T$ differs significantly in growth substrates and its relation to elemental sulfur and H$_2$. In contrast to other Desulfurococcus species, which prefer proteinaceous substrates, it grows well on different monomeric and polymeric carbohydrates. Elemental sulfur is not required for growth, which is in agreement with the lack of an inhibitory effect of H$_2$ on growth.

Peptides are the common substrates of anaerobic, organotrophic, hyperthermophilic archaea, while carbohydrates are metabolized by a limited number of hyperthermophilic archaeal species (Schönheit & Schäfer, 1995). Pyrococcus furiosus can ferment maltose and cellobiose (Fiala & Stetter, 1986; Schäfer & Schönheit, 1992), Pyrococcus woesei, D. amylolyticus, Thermococcus stetteri and Acidilobus aceticus were able to grow fermentatively on starch (Zillig et al., 1987; Bonch-Osmolovskaya et al., 1988; Miroshnichenko et al., 1989; Prokofeva et al., 2000) and Pyrococcus glycorovans was able to grow fermentatively on glucose, cellobiose and starch (Barbier et al., 1999). The range of growth

---

**Fig. 3.** Phylogenetic tree generated by the neighbour-joining method on the basis of 16S rRNA gene sequences showing the position of *Desulfurococcus fermentans* sp. nov. Z-1312$^T$ among members of the family *Desulfurococaceae*. Bootstrap values (of 100 replications) are shown at branch points; values greater than 95 % were considered significant. Bar, 10 nucleotides substitutions per 100 nucleotides.
**Table 1. Characteristics of strain Z-1312\textsuperscript{T} and species of the genus Desulfurococcus**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>D. mucus</th>
<th>D. mobilis</th>
<th>D. amyloyticus</th>
<th>Z-1312\textsuperscript{T}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shape and size of cells</td>
<td>Cocci</td>
<td>Cocci</td>
<td>Irregular cocci</td>
<td>Regular cocci, 1–4 (\mu) m</td>
</tr>
<tr>
<td>Flagellation</td>
<td>No flagella</td>
<td>One flagellum</td>
<td>No flagella</td>
<td>One flagellum</td>
</tr>
<tr>
<td>Growth temperature ((\°)C) (min./opt./max.)</td>
<td>ND/85/ND</td>
<td>ND/85/ND</td>
<td>68/90–92/97</td>
<td>63/80–82/89</td>
</tr>
<tr>
<td>Growth pH (min./opt./max.)</td>
<td>4–5/6–0/7/0</td>
<td>4–5/6–0/7/0</td>
<td>5–7/6–4/7/5</td>
<td>4–8/6–0/6–8</td>
</tr>
<tr>
<td>Growth substrates</td>
<td>Peptides</td>
<td>Peptides</td>
<td>Peptides, starch</td>
<td>Peptides, monosaccharides, polysaccharides including cellulose</td>
</tr>
<tr>
<td>Influence of sulfur on growth</td>
<td>Stimulating</td>
<td>Stimulating</td>
<td>Stimulating</td>
<td>No effect</td>
</tr>
<tr>
<td>Influence of hydrogen on growth</td>
<td>Inhibiting*</td>
<td>ND</td>
<td>Inhibiting*</td>
<td>No effect</td>
</tr>
<tr>
<td>G+C content of DNA (mol%)</td>
<td>51.3</td>
<td>50.8</td>
<td>41.2</td>
<td>42.5</td>
</tr>
</tbody>
</table>

ND, No data.

*Data from Slobodkin & Bonch-Osmolovskaya (1994).

substrates of strain Z-1312\textsuperscript{T} is much wider than that of other hyperthermophilic archaea, including cellulose and other mono-, di- and polysaccharides. In this capacity it resembles ‘*Desulfurococcus saccharovorans*’ (Stetter, 1986); however, the name of this species was never validly published.

A unique feature of the new isolate is its ability to grow on cellulosic substrates (microcrystalline cellulose, carboxymethyl cellulose, filter paper). To our knowledge, strain Z-1312\textsuperscript{T} is the first hyperthermophilic archaeon able to grow on such substrates. So far, genes for endoglucanase that could be involved in cellulose degradation have been found in several hyperthermophilic archaea. Endoglucanase genes detected in the genomes of *P. furiosus*, *Pyrococcus horikoshii* and *Sulfolobus solfataricus* have been cloned and expressed in *Escherichia coli* (Bauer et al., 1999; Limauro et al., 2001; Ando et al., 2002). However, growth of these microorganisms on cellulosic substrates has never been reported. Taking into consideration both phenotypic and genotypic characteristics, the type strain is AY264344.

### Emended description of genus Desulfurococcus Zillig and Stetter 1983

*Desulfurococcus* (De.sul.fu.ro.coc'cus. N.L. pref. de from; L. n. sulfur sulfur; Gr. n. coccus berry; N.L. masc. n. Desulfurococcus the sulfur-reducing coccus).

Archaea of the kingdom *Crenarchaeota*. Cells are regular or irregular cocci, with or without flagella, with a cell envelope of globular structure. Hyperthermophiles with optimum growth temperature of 80–90 °C. Neutrophiles or moderate acidophiles with optimum pH for growth of 6·0–6·5. Obligate anaerobes. Organotrophs utilizing a wide range of organic substrates: peptides and monomeric and polymeric carbohydrates. Fermentative type of metabolism. Growth of representatives of some species is inhibited by molecular hydrogen and stimulated by the presence of elemental sulfur. The G+C content of the DNA is 41–51 mol%. Reported species inhabit terrestrial hot springs. The type species is *Desulfurococcus mucus* Zillig and Stetter 1983 (type strain ATCC 35584\textsuperscript{T} = DSM 2162\textsuperscript{T} = JCM 9187\textsuperscript{T}).

### Acknowledgements

This work was supported by RFBR grants number 02–04–48112 and 03–04–49000, by INTAS grant 01–250 and by the Programs of the Russian Academy of Sciences ‘Molecular and Cell Biology’, ‘Biodiversity’ and ‘Evolution of Biosphere’.

**Description of Desulfurococcus fermentans sp. nov.**


Cells are cocci, 1–4 \(\mu\) m in diameter, with one polar flagellum. Obligate anaerobe. Temperature growth range from 63 to 89 °C, with optimum at 82 °C. pH growth range from 4·8 to 6·8, with optimum at pH 6·0. Obligate chemoorganoheterotroph; obtains energy by fermentation of arabinose, agarose, amylodalin, arbutin, casein hydrolysate, cellulose, dextran, dulcitol, fructose, lactose, laminarin, lichenan, maltose, pectin, peptone, ribose, starch and sucrose. No growth is observed on glucose, mannitol, sorbitol or xylose. Fermentation products detected are acetate, H\(_2\) and CO\(_2\). Growth is not inhibited by 100 % H\(_2\). Elemental sulfur, sulfate, thiosulfate and nitrate do not stimulate growth and are not used as electron acceptors. The G+C content of DNA of the type strain is 42·5 mol%.

The type strain, strain Z-1312\textsuperscript{T} (= DSM 16532\textsuperscript{T} = VKM V-2316\textsuperscript{T}), was isolated from a hot spring of Uzon caldera, Kamchatka peninsula, Russia. The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of the type strain isAY264344.
References


