Thermanaerovibrio velox sp. nov., a new anaerobic, thermophilic, organotrophic bacterium that reduces elemental sulfur, and emended description of the genus Thermanaerovibrio


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A moderately thermophilic, organotrophic bacterium with vibrioid cells was isolated from a sample of a cyanobacterial mat from caldera Uzon, Kamchatka, Russia, and designated strain Z-9701T. Cells of strain Z-9701T were curved, Gram-negative rods, 0.5–0.7 × 2.5–5.0 µm in size, with tapering ends and with fast, wavy movement by means of lateral flagella located on the concave side of the cell. Colonies were small, white, irregular or round, 0.2 mm in diameter, and with even edges. Strain Z-9701T was an obligate anaerobe with a temperature optimum at 60–65 °C and a pH optimum at 7.3. It fermented glucose, fructose, mannose, N-acetyl-D-glucosamine, adonite, arginine, serine, peptone, yeast extract and Casamino acids. The fermentation products formed during growth on glucose were acetate, lactate, H₂, CO₂ and ethanol. Strain Z-9701T reduced elemental sulfur to H₂S during organotrophic growth with glucose or peptides as energy and carbon sources. In the presence of S⁰, strain Z-9701T was capable of lithotrophic growth with molecular hydrogen as energy substrate and 0.1 g yeast extract l⁻¹ as carbon source. Sulfate, thiosulfate, nitrate, Fe(III) and sulfite were not reduced and did not stimulate growth. The G+C content of strain Z-9701T DNA was 54.6 mol %. The results of 16S rDNA sequence analyses revealed that strain Z-9701T belongs to the cluster within the Clostridium group formed by Thermanaerovibrio acidaminovorans, Dethiosulfovibrio peptidovorans, Anaerobaculum thermonerrenum and Aminobacterium colombiense, but the level of sequence similarity with the members of this cluster was not very high (87.6–92.2%). Among these organisms, Thermanaerovibrio acidaminovorans is phenotypically close to strain Z-9701T. However, the two organisms showed a relatively low level of similarity of their 16S rRNA sequences (92.2%) and of DNA–DNA hybridization (15±1%). Nevertheless, on the basis of the similar morphology and physiology of the new isolate and Thermanaerovibrio acidaminovorans, strain Z-9701T was placed in the genus Thermanaerovibrio and a new species, Thermanaerovibrio velox, proposed for it. The type strain is Z-9701T (= DSM 12556T).

Keywords: vibrio, thermophile, organotroph, S⁰ reduction, Thermanaerovibrio velox

INTRODUCTION

Anaerobic organisms with vibrioid cells are either sulfate (thiosulfate)-reducing bacteria or fermentative organotrophs. In total, they include nine genera, of which two comprise thermophilic bacteria. A thermophilic, vibrioid, sulfate-reducing strain was isolated from a hot spring in Yellowstone National Park and characterized as a new genus and species, Thermo-
desulfovibrio yellowstonii (Henry et al., 1994). An organotrophic, thermophilic vibrio isolated from an anaerobic digester system was described as a new species of the genus Selenomonas. Selenomonas acidaminovorans (Guangsheng et al., 1992). This organism grew in co-culture with Methanobacterium thermautotrophicum and was capable of fermenting diverse organic substrates, but no information was given on its ability to reduce inorganic electron acceptors. Recently, based on the comparison of 16S rRNA sequences, Selenomonas acidaminovorans was reclassified as a new genus, Thermanaerovibrio acidaminovorans (Baena et al., 1999). From a cyanobacterial mat from caldera Uzon (Kamchatka, Russia) we isolated an association of two thermophilic microorganisms, both with vibrioid cells, which grew on lactate in the presence of sulfate. The objective of this work was to isolate and identify the components of this association.

**METHODS**

**Environmental samples.** Samples of a cyanobacterial mat developing in a hot spring in caldera Uzon, Kamchatka, Russia, served as the source for enrichment and isolation of anaerobic thermophiles. The pH of the water in the sampling site was 6.8 and its temperature was 65°C.

**Strains.** Thermodesulfovibrio yellowstonii ATCC 51303T was obtained from the ATCC, Manassas, VA, USA; Thermanaerovibrio acidaminovorans DSM 6589T was obtained from the DSMZ, Braunschweig, Germany.

**Media and cultivation.** The initial enrichment was obtained using anaerobically prepared medium of the following composition (g l⁻¹): NH₄Cl, 0.33; KH₂PO₄, 0.33; MgCl₂ 6H₂O, 0.33; CaCl₂ 6H₂O, 0.33; KCl, 0.33; yeast extract, 0.1; Na₂S 9H₂O, 0.5; NaHCO₃, 0.7; Na₂SO₄, 2; resazurin, 0.001. Sodium lactate (50% solution; 10 ml l⁻¹); trace element solution (Kevbrin & Zavarzin, 1992; 1 ml l⁻¹); and vitamin solution (Wolin et al., 1963; 1 ml l⁻¹) were also added.

The pH was maintained at 7.0 with a CO₂/sodium bicarbonate buffer. The medium was dispensed into 15 ml Hungate tubes with screw caps and the head space (10 ml) was filled with a Na₂/C₃O₂ (8:2, v/v) gas mixture. Inoculated tubes were incubated at 55°C. Pure cultures were obtained using the same medium by serial tenfold dilutions with subsequent isolation of single colonies in roll-tubes. For the roll-tubes, Bacto-agar (2.0 g l⁻¹) was added to the medium. A pure culture of the sulfate-reducing isolate was obtained on a medium of the same composition except that sodium pyruvate (3 g l⁻¹) was added instead of lactate. The fermentative strain was isolated on the same medium, but glucose (3 g l⁻¹), yeast extract (0.25 g l⁻¹) and peptone (Difco) (0.25 g l⁻¹) were added as substrate and sources of growth factors, and lactate and sulfate were omitted.

**Physiological studies of the fermentative isolate.** Utilization of various electron acceptors was tested on the same medium as used for isolation, but devoid of sulfate. Possible electron acceptors were added at the following concentrations (mM): sulfate, 10; thiosulfate, 10; sulfite, 1; nitrate, 10. Elemental sulfur (as sulfur flowers) was also tested at a concentration of 1% (w/v). Tests for growth with ferric iron as an electron acceptor were done in sulfide-free medium. Fe(III) was provided in the form of amorphous Fe(III) oxide at a concentration of 90 mM Fe(III) (Slobodkin et al., 1997). No reducing agent was added to the medium. The pH of the autoclaved medium containing Fe(III) was 6.8–6.9. Organic growth substrates, when tested, were added instead of glucose at a concentration of 0.3% (w/v). In positive cases, three subsequent transfers on the same medium were performed.

Lithotrophic growth with molecular hydrogen was tested on medium with the same mineral composition and with yeast extract (0-1 g⁻¹) as the only organic addition. Elemental sulfur served as electron acceptor. Cultivation was performed in 50 ml bottles with screw caps, containing 10 ml of the medium. Head space (40 ml) was filled with 100% hydrogen.

Type strains of Thermodesulfovibrio yellowstonii and Thermanaerovibrio acidaminovorans were grown on the media described in the original publications (Henry et al., 1994; Guangsheng et al., 1992). The ability of Thermanaerovibrio acidaminovorans to reduce sulfur lithotrophically and heterotrophically was tested on the same medium with glucose and hydrogen as growth substrates and 1% elemental sulfur.

Temperature, pH and NaCl concentration ranges for growth were determined in the basal medium with glucose, yeast extract and peptone. The pH range for growth was determined at 60°C.

**Morphological and ultrastructural studies.** The morphology of cells was studied with a Reichert Zetopan anoptopal microscope. Phase-contrast micrographs of bacteria were taken using agar-coated slides (Pfenning & Wagner, 1986). To prepare whole-cell specimens for electron microscopy, cells were sedimented by centrifugation, resuspended in tap water and negatively stained with 1% (w/v) phosphotungstic acid pH 7. For ultrastructural studies, cells were prefixed with glutaraldehyde in culture medium for 30 min at ambient temperature, centrifuged, washed once with 0.15 M potassium phosphate buffer pH 7.2, fixed with 1% (v/v) OsO₄ in acetate/Veronal buffer pH 7.2 for 18 h at 4°C, dehydrated and embedded in Epon 812 by standard methods. They were thin-sectioned on a LKB-4800 ultramicrotome and electron microscopy was performed with a JEM-100C microscope.

**Analytical methods.** Growth was followed by measuring the turbidity of medium in Hungate tubes at 600 nm with a Specol-10 spectrophotometer (Carl Zeiss). Glucose was quantified by the phenol-H₂SO₄ reaction (Hansson & Phillips, 1981). Volatile fermentation products were determined on a Chron-5 (Czechia) gas chromatograph with a flame-ionization detector using argon as carrier gas and a 0.9 m x 3 mm column filled with Chromosorb 101. Hydrogen and carbon dioxide were measured by an LKhM-80 gas chromatograph (Gasochrom) equipped with a thermal conductivity detector. Hydrogen sulfide was measured by the methylene blue colorimetric method (Trüper & Schlegel, 1964).

**Determination of DNA G + C content.** DNA was isolated and purified from lysozyme- and SDS-treated cells by the method of Marmur (1961). The G + C content was determined by the thermal denaturation method (Owen et al., 1969). Escherichia coli K-12 DNA was used as a standard. To determine DNA–DNA hybridization with the type species, Thermanaerovibrio acidaminovorans DNA was immobilized on membrane filters and reassessed under optimal conditions.
conditions (6× SSC, 73 °C) for 48 h. Reference DNA was obtained using a ‘nick-translation’ reaction based on [³H]cytidine (Rigby et al., 1977).

16S rDNA sequence determination and analysis. 16S rDNA was selectively amplified from genomic DNA by PCR using 5’-AGAGTTTGATCCTGGCTCAG-3’ as the forward primer and 5’-TACGTTACCTGTTACGACTT-3’ as the reverse primer (Lane, 1991). The PCR reaction was carried out in 100 µl of a reaction mixture containing 1 µg of DNA template, 200 µM (each) primers, 200 µM (each) DNPs and 3 units Tet-z polymerase (BioMaster) in reaction buffer (100 mM Tris/HCl pH 8.3, 500 mM KCl, 20 mM MgCl₂). Temperature cycling was done by using 30 amplification cycles of 1 min at 94 °C, 1 min at 42 °C and 1 min at 72 °C. The final extension was carried out at 72 °C for 6 min. The PCR products were purified using the PCR-prep kit (Promega) as recommended by the manufacturer. The 16S rDNA was sequenced in both directions by using forward and reverse universal primers. DNA sequencing was performed by using Sequenase version 2 of the VSB kit (USB).

The sequence was pre-aligned with eubacterial sequences obtained from the Ribosomal Database Project. It was then pre-aligned with a representative set of 16S rDNA sequences obtained from the Ribosomal Database Project and from recent GenBank releases by using MULTALIGN software (Corpet, 1988). Positions of sequence and alignment uncertainties were omitted and in total 1125 nucleotides were used in the analysis. Pairwise evolutionary distances were computed by using the correction of Jukes & Cantor (1969) and transversions only (Swofford & Olsen, 1990). The unrooted phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) with bootstrap analysis of 100 trees using the programs of the TREECON package (Van de Peer & De Wachter, 1994).

Nucleotide sequence accession numbers. The GenBank accession number of the 16S rDNA sequence of strain Z-9701T is AF161069. The accession numbers of the sequences used as references are as follows: Caldicellulosiruptor owensensis OL7, U80596; Dictyoglomus thermophilum H-6-12, X69194; Desulfotobacterium dehalogenans JW/IIU-DC13, U40078; Desulfotomaculum nigrificans NCIMB 8395T, X62176; Moorella thermoacetica LDLT, X58352; Thermoanaerobacter ethanolicus JW-200T, L09162; Anaerobranca horikoshii JW/YL-138T, U21809; Thermoanaerobacterium thermosulfurigenes E100-69T, L09161; Dethiosulfovibrio peptidovorans G4207T, U52817; Thermoterrabacterium ferrireducens JW/AS-Y7T, U76363; Sporomusa paucivorans DSM 3637T, M59117; Thermoanaerobacterium acidaminovorans DSM 6589T, AF071414; Anaerobaculum thermoterrenum RWcitT, U50711; Aminobacterium colombiensis ALA-1T, AF069287.

RESULTS

Enrichment and isolation

An enrichment culture of sulfate-reducing bacteria was obtained in anaerobically prepared medium containing sulfate and lactate. After 5 d incubation at 55 °C, two micro-organisms, one small and one large, with vibrioid cells dominated in the medium in an approximate ratio of 3:1; both were highly motile. After inoculation into the same medium solidified with agar, colonies of two types appeared: small, white, oval colonies 0.1–0.3 mm in diameter with even edges and dense black centres; and small, white, irregular or round colonies 0.2 mm in diameter with even edges. Both organisms were isolated in pure culture.

The colonies of the first type contained small, vibrioid cells. This strain was designated Z-9702. It showed 92% DNA–DNA hybridization with the type strain of Thermodesulfovibrio yellowstonii, ATCC 51303T (Henry et al., 1994) and was identified as a strain of this species. Unlike ATCC 51303T, Z-9702 did not grow in
a medium containing lactate (3 g l\(^{-1}\)) and sulfate. Its growth was supported by pyruvate (3 g l\(^{-1}\)), formate (3 g l\(^{-1}\)) and molecular hydrogen.

Colonies of the second type contained large, vibrioid cells. This organism was designated strain Z-9701\(^{\text{T}}\). When grown in liquid medium with sulfate and lactate, isolate Z-9701\(^{\text{T}}\) exhibited only weak growth and no H\(_2\)S production. After transfer to medium containing glucose, growth of isolate Z-9701\(^{\text{T}}\) became much better. For further experiments, glucose-containing medium was used.

**Morphology and ultrastructural studies**

Cells of strain Z-9701\(^{\text{T}}\) were curved rods with tapering ends, occurring singly or in pairs, and showing fast, wave-like movement (Fig. 1a). The size of the cells varied within the range 0.5–0.7 \(\times\) 2.5–5.0 \(\mu\)m (depending on the age of the culture). Formation of spores was never observed. The organism multiplied by binary fission (see arrow in Fig. 1a). Electron microscopy of the negatively stained cells revealed lateral flagella located on the concave side of the cell (Fig. 1b). Ultrathin sections showed a typical Gram-negative cell envelope profile with a multilayered cell wall (Fig. 2).

**Growth characteristics**

Strain Z-9701\(^{\text{T}}\) was obligately anaerobic and grew only after reduction of the medium with sodium sulfide. Growth of strain Z-9701\(^{\text{T}}\) occurred at temperatures from 45 to 70 °C, with an optimum between 60 and 65 °C (Fig. 3a). The pH optimum for growth was at 7.3; no growth was obtained at pH 4.5 or pH 8.0 (Fig. 3b). NaCl was not required for growth. Growth occurred at NaCl concentrations of up to 35 g l\(^{-1}\).

Strain Z-9701\(^{\text{T}}\) was able to grow by fermentation of glucose, fructose, mannose, \(N\)-acetyl-\(D\)-glucosamine, adonite, arginine, serine, peptone, yeast extract and Casamino acids. No growth was observed on galactose, \(D\)-ribose, sorbose, \(D\)-xylose, \(D\)-cellobiose, \(D\)-maltose, \(D\)-lactose, melibiose, raffinose, sucrose, trehalose, acetate, ascorbate, butyrate, citrate, formate, glycolate, glutamate, lactate, malate, pyruvate, propionate, succinate, tartrate, \(L\)-dulcitol, \(L\)-inositol, ethanol, mannitol, methanol, propanol, \(L\)-sorbitol, betaine, \(L\)-histidine, glycerol, glycogen, \(D\L\)-lysine, sarcosine, tryptone, choline, cellulose, chitin, starch or molecular hydrogen (in the absence of elemental sulfur).

Fermentation products formed during growth on
The presence of elemental sulfur, strain Z-9701
*T found that the organism could grow lithotrophically
lithotrophic growth with molecular hy-
Thermanaerovibrio acidaminovorans
Thermanaerovibrio velox sp. nov.
Table 1. Growth of strain Z-9701\(^T\) and Thermanaerovibrio acidaminovorans in the presence and absence of elemental sulfur

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate (3 g l(^{-1}))</th>
<th>Growth rate without S(^0) (d(^{-1}))</th>
<th>Growth with S(^0)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Growth rate (d(^{-1}))</td>
</tr>
<tr>
<td>Strain Z-9701(^T)</td>
<td>Peptone</td>
<td>0.24</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Yeast extract</td>
<td>0.26</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Tryptase</td>
<td>ND</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Casamino acids</td>
<td>0.43</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.64</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>H(_2) in the presence of</td>
<td>No growth</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>yeast extract (0.1 g l(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H(_2)</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Control*</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>H(_2) in the presence of</td>
<td>No growth</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td>yeast extract (0.1 g l(^{-1}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>H(_2)</td>
<td>No growth</td>
<td>No growth</td>
</tr>
<tr>
<td></td>
<td>Control*</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>

Thermanaerovibrio acidaminovorans DSM 6589\(^T\) was tested for the ability to reduce elemental sulfur. Sulfur (1%) inhibited the growth of Thermanaerovibrio acidaminovorans on the medium with glucose, but it was found that the organism could grown lithotrophically with H\(_2\) and S\(^0\) (Table 1).

DNA analysis

The G+C content of strain Z-9701\(^T\) DNA was 54.6 mol%. DNA-DNA hybridization with Thermanaerovibrio acidaminovorans DSM 6589\(^T\) was 15±1%.

Phylogenetic analysis

The almost complete sequence of the 16S rDNA (1476 nucleotides) of strain Z-9701\(^T\) covering the region between positions 8 and 1494 (E. coli numbering) was determined. A preliminary phylogenetic analysis performed with representatives of the domain Bacteria revealed that the new isolate Z-9701\(^T\) was a member of the Bacillus–Clostridium subphylum of the Gram-positive bacteria. Several phylogenetic trees were constructed by changing the spectrum of reference organisms. These trees demonstrated that strain Z-9701\(^T\) was a member of Clostridium group which includes at least 19 defined clusters and several lines of descent (Collins et al., 1994). A final comparison of 1125 nucleotides of the 16S rDNA sequences of strain Z-9701\(^T\) and 16 reference strains of the Clostridium group was carried out and used for reconstruction of a phylogenetic tree (Fig. 4) and calculation of sequence similarity. The tree showed strain Z-9701\(^T\) to form a monophyletic cluster (92.2% sequence similarity, bootstrap value 99%) with Thermanaerovibrio (Selenomonas) acidaminovorans (Guangsheng et al., 1992; Baena et al., 1999). More distant relatedness was found with Aminobacterium colombiense (Baena et al., 1998) (89.6% sequence similarity), Dethiosulfovibrio peptidovorans (Magot et al., 1997) (88.0% sequence similarity) and Anaerobaculum thermoterrreum (Rees et al., 1997) (87.6% sequence similarity). These organisms formed with strain Z-9701\(^T\) a new cluster of the Clostridium group with the highest level of bootstrap probability (100%). This cluster was peripherally
related to cluster V of the Clostridium group (Collins et al., 1994), consisting of the genus Thermoanaerobacter, with a level of sequence similarity in the range 88.0–88.8%. The level of 16S rDNA sequence similarity between strain Z-9701T and other members of the Clostridium group analysed was 83.1–86.6%. A direct comparison of 1468 nucleotides of the 16S rDNA sequence of strain Z-9701T with those of its closest relative, Thermanaerovibrio acidaminovorans, was carried out and the level of sequence similarity was found to be 92.2%.

DISCUSSION

Production of organic matter in thermophilic cyanobacterial mats is accompanied by its efficient destruction, mostly anaerobic (Ward et al., 1984; Bonch-Osmolovskaya et al., 1987). Cyanobacterial mats have served as the source for isolation of many new thermophilic prokaryotes, among them organotrophic anaerobes (Lowe et al., 1993), methanogens (Zeikus et al., 1980; Nozhevnikova & Yagodina, 1982), and sulfate- and sulfur-reducing bacteria (Zeikus et al., 1983; Bonch-Osmolovskaya, 1994). The latter group comprised thermophilic, sulfur-reducing bacteria of different metabolic types: lithotrophic sulfur-respiring bacteria of the genus Desulfuromonas (Bonch-Osmolovskaya et al., 1990; Miroshnichenko et al., 1998) and anaerobic organotrophs which reduced elemental sulfur during the course of fermentation and belonged to the genus Thermoanaerobacter (Bonch-Osmolovskaya et al., 1997).

A lactate-utilizing, sulfate-reducing enrichment obtained from a cyanobacterial mat was found to contain two forms of micro-organism, both with vibrioid cells. In pure culture neither of them was capable of sulfate reduction with lactate as growth substrate. It might therefore be assumed that the two organisms formed a syntrophic association, in which one organism was producing hydrogen from lactate and the other one used the hydrogen for sulfate reduction. Indeed, the organism with smaller cells, strain Z-9702, was identified as Thermodesulfovibrio yellowstonii (Henry et al., 1994), based on the high level of DNA–DNA hybridization (92%) with the type strain, ATCC 51303T. Strain Z-9702 differed from ATCC 51303T in its inability to grow on lactate-containing medium. Its only growth substrates were molecular hydrogen, formate and pyruvate. Growth and sulfate reduction on lactate-containing medium was possible for strain Z-9702 only in co-culture with strain Z-9701T, which produced molecular hydrogen from lactate.

An organism with larger cells (strain Z-9701T) was found to be an organotroph, fermenting numerous organic substrates. It was also found that elemental sulfur stimulated its growth on fermentable substrates—a phenomenon described previously for many organotrophic, thermophilic prokaryotes (Fiala & Stetter, 1986; Bonch-Osmolovskaya & Miroshnichenko, 1994; Slobodkin & Bonch-Osmolovskaya, 1994; Bonch-Osmolovskaya et al., 1997). Strain Z-9701T differs from other organotrophic, sulfur-reducing thermophiles in its ability to grow lithotrophically with molecular hydrogen and elemental sulfur. The original description of Thermoclostridium acidaminovorans (formally Selenomonas acidaminovorans) includes reference to an inhibitory effect of elemental sulfur on its growth on glucose (Guangsheng et al., 1992). However, we found that this organism is able to grow lithotrophically with molecular hydrogen and elemental sulfur. We consider this feature to be an important characteristic of the genus Thermaanaerobacter. Recently, the widespread ability of thermophilic prokaryotes to reduce ferric iron lithotrophically was reported (Vagras et al., 1998; Slobodkin et al., 1999). Our finding prompts the
Strain Z-9701 our new isolate, we propose to placing it in the genus similarity of 92% in its ability to reduce elemental sulfur in its ability to reduce elemental sulfur during organotrophic growth and in the stimulating effect sulfur reduction has on its growth. Strain Z-9701 is unable to grow on succinate whilst Thermanaerobacterium acidaminovorans decarboxylates succinate to propionate. Thermanaerobacterium acidaminovorans degrades glucose to acetate and H₂, while strain Z-9701 ferments it to acetate, lactate, CO₂, H₂ and ethanol. Based on its phenotypic and genotypic differences compared to Thermanaerobacterium acidaminovorans, we propose a new species for strain Z-9701, Thermanaerobacterium velox.

**Emended description of genus Thermanaerobacterium** (Baena et al. 1999)

*Thermanaerobacterium* (Therm.an ae.ro.vib’ri.o. Gr. adj. *thermos* hot; Gr. pref. *an* not; Gr. *n. aer* air; M.L. masc. *n. vibrio* that vibrates; M.L. masc. *n. Thermanaerobacterium* a thermophilic vibrating anaerobe).

Strictly anaerobic, curved cells. Motile by means of lateral flagella, located on the concave side of the cell. Gram-negative. Non-spore-forming. Multiplication occurs by binary fission. Thermophilic. Neutrophilic. Grows chemo-organotrophically with fermentable substrates or lithoheterotrophically with molecular hydrogen and elemental sulfur, reducing the sulfur to H₂S. The G+C content of the DNA is from 54.5 to 56.5 mol%. Habitats are granular methanogenic sludge and neutral hot springs. The type species is *Thermanaerobacterium acidaminovorans* Su883^T (= DSM 6589^T).

**Description of Thermanaerobacterium velox sp. nov.**


Cells are curved rods, 0.5–0.7 x 2.5–5.0 µm in size, with wave-like movement by means of lateral flagella located on the concave side of the cell. Colonies are small, white, irregular or round, 0.2 mm in diameter, and with an even edge. The cell wall has a Gram-negative structure. Non-spore-forming. Multiplication

**Table 2.** Comparison of phenotypic characteristics of anaerobic bacteria with Gram-negative, curved, rod-shaped cells

<table>
<thead>
<tr>
<th>Character</th>
<th>Genus Selenomonas</th>
<th>Genus Succinivibrio</th>
<th>Thermodesulfovibrio yellowstonii†</th>
<th>Thermanaerobacterium acidaminovorans‡</th>
<th>Strain Z-9701^T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell shape</td>
<td>Curved to helical rods</td>
<td>Curved rods</td>
<td>Curved rods</td>
<td>Curved rods</td>
<td>Curved rods</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>0.9–1.1 x 3.0–6.0</td>
<td>0.4–0.6 x 1.0–7.0</td>
<td>0.3 x 1.5</td>
<td>0.5–0.6 x 2.5–3.0</td>
<td>0.5–0.7 x 2.5–5.0</td>
</tr>
<tr>
<td>Flagellation:</td>
<td>Polar, monotrichous</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lateral tuft on concave side of cell</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Optimum temperature (°C)</td>
<td>37</td>
<td>30–39</td>
<td>65</td>
<td>50–55</td>
<td>60–65</td>
</tr>
<tr>
<td>Type of metabolism:</td>
<td>Fermentative</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sulfate reduction</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glucose fermentation products</td>
<td>Propionate, acetate, lactate, succinate</td>
<td>Succinate, acetate, formate, lactate, CO₂</td>
<td>–</td>
<td>Acetate, H₂</td>
<td>Acetate, lactate, H₂, CO₂, ethanol</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>54–0.61–0</td>
<td>nd</td>
<td>29.5</td>
<td>56.5</td>
<td>54.6</td>
</tr>
</tbody>
</table>

ND, Not determined.

* Data obtained from Holdeman et al. (1984).
† Data obtained from Henry et al. (1994).
‡ Data obtained from Guangsheng et al. (1992).
occurs by binary fission. Growth occurs in a temperature range from 45 to 70 °C, with an optimum between 60 and 65 °C, and in a pH range from 4.5 to 8.0, with an optimum at pH 7.3. Ferments glucose, fructose, mannose, N-acetyl-d-glucosamine, adonite, arginine, serine, peptone, yeast extract and Casamino acids. When grown on glucose, produces acetate, lactate, H₂, CO₂, and ethanol. No growth occurs on galactose, ribose, sorbose, xylose, cellobiose, maltose, lactose, melibiose, raffinose, sucrose, trehalose, acetate, ascorbate, butyrate, citrate, formate, glycolate, glutamate, lactate, malate, pyruvate, propionate, succinate, tartrate, t-dulcitol, t-inositol, ethanol, mannitol, methanol, propanol, t-sorbitol, betaine, t-histidine, glycerol, glycerogen, dl-lysine, sarcosine, tryptophan, choline, cellulose, chitin, starch or molecular hydrogen (in the absence of elemental sulfur). Yeast extract (0.25 g l⁻¹) and peptone (0.25 g l⁻¹) stimulate organotrophic growth on glucose. Yeast extract (0.1 g l⁻¹) is required for lithotrophic growth with H₂ and S⁰. Elemental sulfur is reduced to H₂S during, and stimulates, organotrophic growth with glucose, peptone, yeast extract, trypticase and Casamino acids, or lithotrophic growth with molecular hydrogen. Sulfate, thiosulfate, nitrate, Fe(III) and sulfite are not reduced and do not stimulate growth. The DNA G + C content is 54.6 mol%. The organism was isolated from a thermophilic cyanobacterial mat from caldera Uzon, Kamchatka, Russia. The type strain is Z-9701T (= DSM 12556T).

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