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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-9701^T. Cells of strain Z-9701^T 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-9701^T 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, H_2, CO_2 and ethanol. Strain Z-9701^T reduced elemental sulfur to H_2S during organotrophic growth with glucose or peptides as energy and carbon sources. In the presence of S^0, strain Z-9701^T 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-9701^T DNA was 54·6 mol%. The results of 16S rDNA sequence analyses revealed that strain Z-9701^T belongs to the cluster within the *Clostridium* group formed by *Thermanaerovibrio acidaminovorans*, *Dethiosulfovibrio peptidovorans*, *Anaerobaculum thermoterrenum* 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-9701^T. 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-9701^T was placed in the genus *Thermanaerovibrio* and a new species, *Thermanaerovibrio velox*, proposed for it. The type strain is Z-9701^T (= DSM 12556^T).

Keywords: vibrio, thermophile, organotroph, S^0 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-*

The GenBank accession number for the 16S rDNA sequence of strain Z-9701^T is AF161069.

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 thermoautotrophicum* 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 51303^T was obtained from the ATCC, Manassas, VA, USA; *Thermanaerovibrio acidaminovorans* DSM 6589^T 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}): NH_4Cl, 0·33; KH_2PO_4, 0·33; $MgCl_2.6H_2O$, 0·33; $CaCl_2.6H_2O$, 0·33; KCl, 0·33; yeast extract, 0·1; $Na_2S.9H_2O$, 0·5; $NaHCO_3$, 0·7; Na_2SO_4, 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 CO_2/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 N_2/CO_2 (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 anoptral microscope. Phase-contrast micrographs of bacteria were taken using agar-coated slides (Pfennig & 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) phosphotungstate 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) OsO_4 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-H_2SO_4 reaction (Hansson & Phillips, 1981). Volatile fermentation products were determined on a Chrom-5 (Czechia) gas chromatograph with a flame-ionization detector using argon as carrier gas and a 0·9 m × 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 lyzosyme- 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 reassociated under optimal

conditions (6 × SSC, 73 °C) for 48 h. Reference DNA was obtained using a 'nick-translation' reaction based on [^{3}H]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′-TACGGTTACCTTGTTACGACTT-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 $MgCl_2$). 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 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-9701^{T} is AF161069. The accession numbers of the sequences used as references are as follows: *Caldicellulosiruptor owensensis* OL^{T}, U80596; *Dictyoglomus thermophilum* H-6-12^{T}, X69194; *Desulfitobacterium dehalogenans* JW/IU-DC1^{T}, U40078; *Desulfotomaculum nigrificans* NCIMB 8395^{T}, X62176; *Moorella thermoacetica* LJDT, X58352; *Thermoanaerobacter ethanolicus* JW-200^{T}, L09162; *Anaerobranca horikoshii* JW/YL-138^{T}, U21809; *Thermoanaerobacterium thermosulfurigenes* E100-69^{T}, L09161; *Dethiosulfovibrio peptidovorans* G4207^{T}, U52817; *Thermoterrabacterium ferrireducens* JW/AS-Y7^{T}, U76363; *Sporomusa paucivorans* DSM 3637^{T}, M59117; *Thermanaerovibrio acidaminovorans* DSM 6589^{T}, AF071414; *Anaerobaculum thermoterrenum* RWcit^{T}, U50711; *Aminobacterium colombiense* ALA-1^{T}, AF069287.

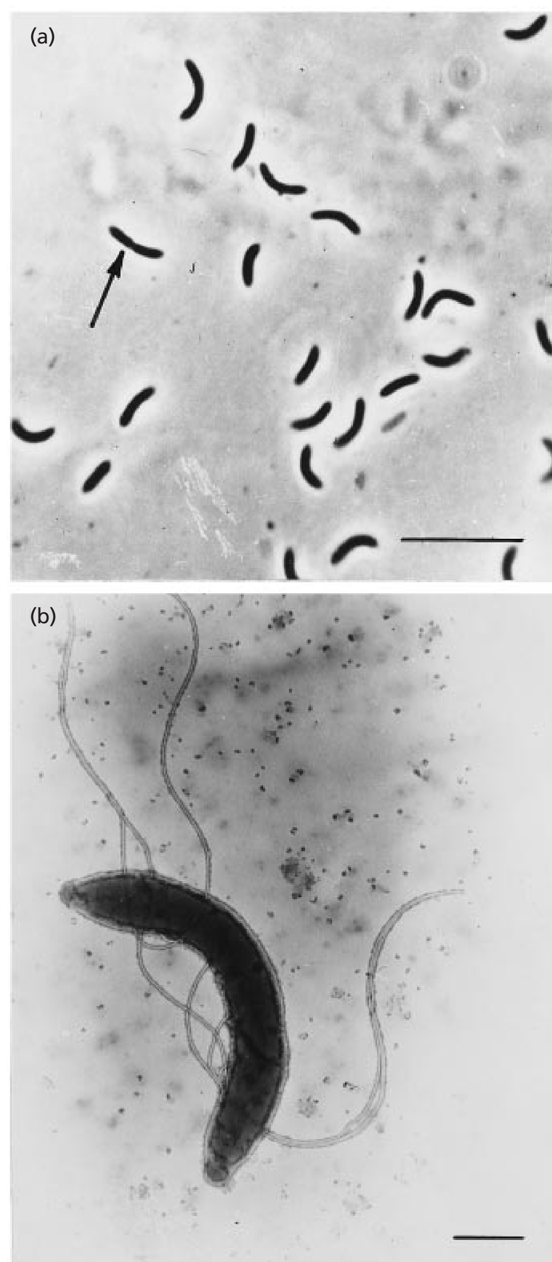

Fig. 1. Morphology of strain Z-9701^{T}. (a) Cells as viewed under a phase-contrast microscope. Bar, 10 μm. (b) Negatively stained cell with flagella located on the concave side. Bar, 1 μm.

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 51303^{T} (Henry *et al.*, 1994) and was identified as a strain of this species. Unlike ATCC 51303^{T}, Z-9702 did not grow in

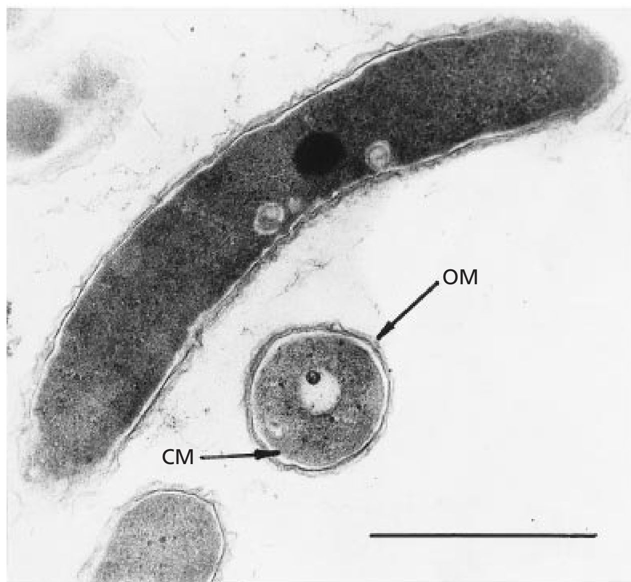

Fig. 2. Ultrastructure of strain Z-9701^T. CM, cytoplasmic membrane; OM, outer membrane. Bar, 1 μm.

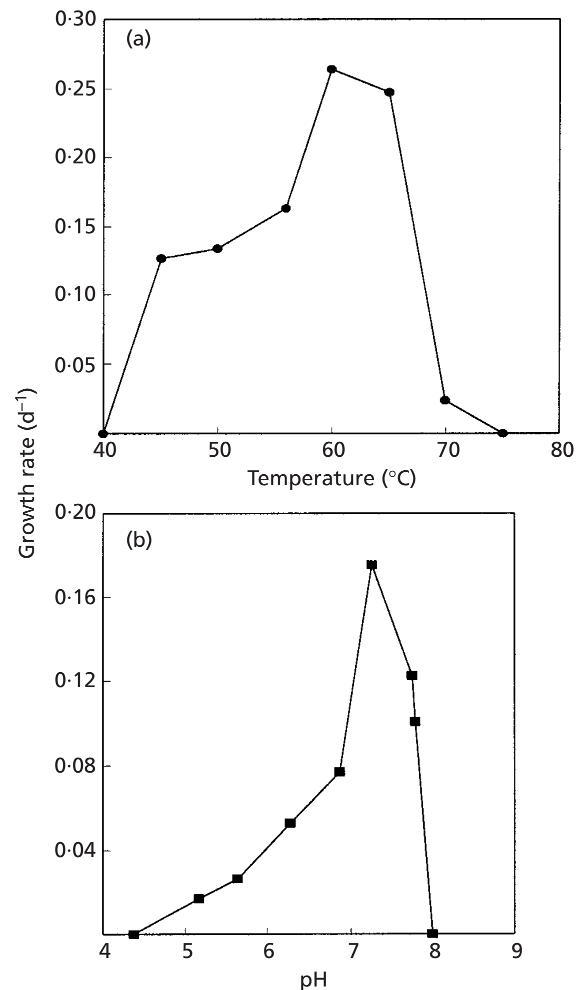

Fig. 3. (a) Effect of temperature on the growth of strain Z-9701^T. (b) Effect of pH on the growth of strain Z-9701^T. The pH was determined at 60 °C and initial pH values are plotted.

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^T. When grown in liquid medium with sulfate and lactate, isolate Z-9701^T exhibited only weak growth and no H_2S production. After transfer to medium containing glucose, growth of isolate Z-9701^T became much better. For further experiments, glucose-containing medium was used.

Morphology and ultrastructural studies

Cells of strain Z-9701^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 × 2·5–5·0 μm (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^T was obligately anaerobic and grew only after reduction of the medium with sodium sulfide. Growth of strain Z-9701^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^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, DL-lysine, sarcosine, tryptone, choline, cellulose, chitin, starch or molecular hydrogen (in the absence of elemental sulfur).

Fermentation products formed during growth on

Table 1. Growth of strain Z-9701[T] and *Thermanaerovibrio acidaminovorans* in the presence and absence of elemental sulfur

Organism	Substrate (3 g l^{-1})	Growth rate without S^0 (d^{-1})	Growth with S^0	
			Growth rate (d^{-1})	H_2S formed (mM l^{-1})
Strain Z-9701[T]	Peptone	0·24	0·40	8·2
	Yeast extract	0·26	0·37	7·2
	Trypticase	ND	0·35	6·2
	Casamino acids	0·43	0·50	5·3
	Glucose	0·64	1·15	3·5
	H_2 in the presence of yeast extract (0·1 g l^{-1})	No growth	0·20	13·1
	H_2	No growth	No growth	0
	Control*	No growth	No growth	0
Thermanaerovibrio acidaminovorans	Glucose	No growth	No growth	0
	H_2 in the presence of yeast extract (0·1 g l^{-1})	No growth	0·10	5·1
	H_2	No growth	No growth	0
	Control*	No growth	No growth	0

ND, Not determined.

* No substrate, 0·1 g yeast extract l^{-1}.

glucose were acetate, lactate, CO_2, H_2 and ethanol. Utilization of 10 mM glucose resulted in production of 7 mM H_2, 2·3 mM ethanol and 5·3 mM acetate.

Elemental sulfur was found to stimulate the growth rate of strain Z-9701[T] approximately twofold on glucose, peptone, yeast extract, trypticase and Casamino acids (Table 1). Concurrently with growth, elemental sulfur was reduced to hydrogen sulfide. In the presence of elemental sulfur, strain Z-9701[T] was capable of lithotrophic growth with molecular hydrogen; 0·1 g yeast extract l^{-1} was required. Lithotrophic growth of isolate Z-9701[T] was stable and did not decrease after three consequent transfers on the same medium. Sulfate, thiosulfate, nitrate, Fe(III) and sulfite were not reduced and did not stimulate growth. The presence of peptone (0·25 g l^{-1}) and yeast extract (0·25 g l^{-1}) stimulated growth rate and cell yield, the latter being 50 times higher than on glucose.

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 grow 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 thermoterrenum* (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

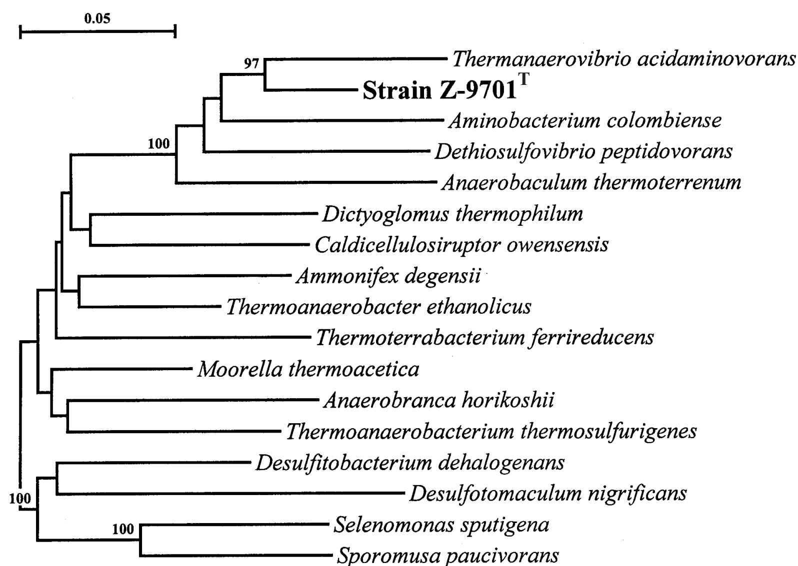

Fig. 4. Unrooted phylogenetic tree showing the position of strain Z-9701^T among members of the *Clostridium* group of the *Bacillus–Clostridium* phylum of the Gram-positive bacteria. Bootstrap values (expressed as a percentage of 100 replications) are shown at branch points; values greater than 95 were considered significant. Scale-bar represents the Jukes–Cantor distance.

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-9701^T 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-9701^T 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 *Desulfurella* (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 51303^T. Strain Z-9702 differed from ATCC 51303^T 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-9701^T, which probably produced molecular hydrogen from lactate.

An organism with larger cells (strain Z-9701^T) 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-9701^T differs from other organotrophic, sulfur-reducing thermophiles in its ability to grow lithotrophically with molecular hydrogen and elemental sulfur. The original description of *Thermanaerovibrio 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 *Thermanaerovibrio*. 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

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

Character	Family *Bacteroidaceae**		*Thermodesulfovibrio yellowstonii*†	*Thermanaerovibrio acidaminovorans*‡	Strain Z-9701^T
	Genus *Selenomonas*	Genus *Succinivibrio*			
Cell shape	Curved to helical rods	Curved rods	Curved rods	Curved rods	Curved rods
Cell size (μm)	0·9–1·1 × 3·0–6·0	0·4–0·6 × 1·0–7·0	0·3 × 1·5	0·5–0·6 × 2·5–3·0	0·5–0·7 × 2·5–5·0
Flagellation:					
Polar, monotrichous	−	+	+	−	−
Lateral tuft on concave side of cell	+	−	−	+	+
Optimum temperature (°C)	37	30–39	65	50–55	60–65
Type of metabolism:					
Fermentative	+	+	−	+	+
Sulfate reduction	−	−	+	−	−
Glucose fermentation products	Propionate, acetate, lactate, succinate	Succinate, acetate, formate, lactate, CO_2	−	Acetate, H_2	Acetate, lactate, H_2, CO_2, ethanol
DNA G+C content (mol%)	54·0–61·0	ND	29·5	56·5	54·6

ND, Not determined.

* Data obtained from Holdeman *et al.* (1984).

† Data obtained from Henry *et al.* (1994).

‡ Data obtained from Guangsheng *et al.* (1992).

suggestion that the ability to grow lithotrophically with hydrogen and elemental sulfur might be shown by a much wider range of organisms than just the highly specialized, sulfur-respiring lithotrophs.

Phenotypically, strain Z-9701^T is rather close to *Thermanaerovibrio* (*Selenomonas*) *acidaminovorans* (Guangsheng *et al.*, 1992; Baena *et al.*, 1999), the only thermophilic, anaerobic organotroph with vibrioid cells described previously. The two organisms have similar morphology and physiology (Table 2), and have a close DNA G+C content, although the level of DNA–DNA hybridization was rather low (15±1%).

Comparison of the 16S rDNA sequences showed that, in spite of its Gram-negative cell wall structure, strain Z-9701^T belongs to the *Bacillus–Clostridium* phylum of the Gram-positive bacteria. Within this phylum, strain Z-9701^T belongs to a new line of descent of the *Clostridium* group which comprises some new Gram-negative genera, *Thermanaerovibrio acidaminovorans*, *Aminobacterium colombiense*, *Dethiosulfovibrio peptidovorans* and *Anaerobaculum thermoterrenum*. The degree of similarity between the 16S rRNA sequences of strain Z-9701^T and its closest relative, *Thermanaerovibrio acidaminovorans*, was found to be at the level of generic differentiation (92·2%). Nevertheless, taking into consideration the significant phenotypic similarity of *Thermanaerovibrio acidaminovorans* and our new isolate, we propose to placing it in the genus *Thermanaerovibrio*.

Strain Z-9701^T differs from *Thermanaerovibrio acidaminovorans* in its ability to reduce elemental sulfur during organotrophic growth and in the stimulating effect sulfur reduction has on its growth. Strain Z-9701^T is unable to grow on succinate whilst *Thermanaerovibrio acidaminovorans* decarboxylates succinate to propionate. *Thermanaerovibrio acidaminovorans* degrades glucose to acetate and H_2, while strain Z-9701^T ferments it to acetate, lactate, CO_2, H_2 and ethanol. Based on its phenotypic and genotypic differences compared to *Thermanaerovibrio acidaminovorans*, we propose a new species for strain Z-9701^T, *Thermanaerovibrio velox*.

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

Thermanaerovibrio (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. *Thermanaerovibrio* 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 H_2S. 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 *Thermanaerovibrio acidaminovorans* Su883^T (= DSM 6589^T).

Description of *Thermanaerovibrio velox* sp. nov.

Thermanaerovibrio velox (ve′lox. L. adj. *velox* quick, fast, motile).

Cells are curved rods, 0·5–0·7 × 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

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, H_2, CO_2 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, L-dulcitol, L-inositol, ethanol, mannitol, methanol, propanol, L-sorbitol, betaine, L-histidine, glycerol, glycogen, DL-lysine, sarcosine, tryptone, 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 H_2 and S^0. Elemental sulfur is reduced to H_2S 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-9701^T (= DSM 12556^T).

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