

# Sulfidogenesis from 2-Aminoethanesulfonate (Taurine) Fermentation by a Morphologically Unusual Sulfate-Reducing Bacterium, *Desulforhopalus singaporensis* sp. nov.

THOMAS J. LIE,<sup>†</sup> MICHAEL L. CLAWSON, WALTER GODCHAUX, AND EDWARD R. LEADBETTER\*

*Department of Molecular and Cell Biology, University of Connecticut, Storrs, Connecticut 06269-2131*

Received 15 January 1999/Accepted 20 May 1999

A pure culture of an obligately anaerobic marine bacterium was obtained from an anaerobic enrichment culture in which taurine (2-aminoethanesulfonate) was the sole source of carbon, energy, and nitrogen. Taurine fermentation resulted in acetate, ammonia, and sulfide as end products. Other sulfonates, including 2-hydroxyethanesulfonate (isethionate) and cysteate (alanine-3-sulfonate), were not fermented. When malate was the sole source of carbon and energy, the bacterium reduced sulfate, sulfite, thiosulfate, or nitrate (reduced to ammonia) but did not use fumarate or dimethyl sulfoxide as a terminal electron acceptor for growth. Taurine-grown cells had significantly lower adenylylphosphosulfate reductase activities than sulfate-grown cells had, which was consistent with the notion that sulfate was not released as a result of oxidative C-S bond cleavage and then assimilated. The name *Desulforhopalus singaporensis* is proposed for this sulfate-reducing bacterium, which is morphologically unusual compared to the previously described sulfate-reducing bacteria by virtue of the spinae present on the rod-shaped, gram-negative, nonmotile cells; endospore formation was not discerned, nor was desulfoviridin detected. Granules of poly- $\beta$ -hydroxybutyrate were abundant in taurine-grown cells. This organism shares with the other member of the genus *Desulforhopalus* which has been described a unique 13-base deletion in the 16S ribosomal DNA. It differs in several ways from a recently described endospore-forming anaerobe (K. Denger, H. Laue, and A. M. Cook, *Arch. Microbiol.* 168:297–301, 1997) that reportedly produces thiosulfate but not sulfide from taurine fermentation. *D. singaporensis* thus appears to be the first example of an organism which exhibits sulfidogenesis during taurine fermentation. Implications for sulfonate sulfur in the sulfur cycle are discussed.

Organosulfur compounds are widely distributed in the environment (25), and the range of oxidation states of sulfur in these compounds (+6 in chondroitin sulfate to -1 in methanethiol) is similar to the range for inorganic sulfur (+6 for sulfate to -2 for hydrogen sulfide) (21, 50). The nomenclature of the organosulfur compounds differs according to the oxidation state of the sulfur (50). Sulfonic acids are a class of organosulfur compounds with the general structure  $R-H_2C-SO_3^-$ , in which the sulfur is at an oxidation state of +5 (21, 47); the R represents a carbon-containing residue which can be aliphatic, aromatic, or more complex. Sulfonates are synthesized by diverse biota (33, 42) and are also synthesized chemically; the sulfonate moiety ( $-SO_3^-$ ) is often added to compounds to increase water solubility (3, 50) or to enhance resistance to biodegradation (28, 32). Sulfonates are especially abundant in some environments (28, 33) and thus may serve as nutrients or sources of energy. The initial focus on the metabolism of sulfonates was primarily on aerobic utilization of these compounds (32, 42); cleavage of the carbon-sulfur bond usually involves monooxygenases (16, 22) and sulfolyases (27). However, recent reports have demonstrated that sulfonates can be mineralized under strictly anaerobic conditions (8, 9, 29, 30, 33, 34). In initial studies of the use of sulfonates as terminal electron acceptors (TEA) by sulfate-reducing bacteria (SRB), we noted that none of the compounds tested served as a sole

source of carbon and energy for growth of strain IC1 (34). Recently, however, cysteate fermentation (29) and taurine fermentation (9) have been described for bacteria belonging to two different genera. Although the sole structural difference between cysteate and taurine is the carboxyl group of cysteate, the end products formed from cysteate fermentation (ammonia, acetate, sulfide, sulfate) differed from the end products formed from taurine fermentation (ammonia, acetate, thiosulfate). Here we describe the ability of a morphologically unusual sulfate-reducing bacterium that ferments taurine to form ammonia, acetate, and sulfide as end products.

## MATERIALS AND METHODS

**Chemicals.** The chemicals used were analytical or reagent grade and were purchased from Fisher Scientific (Pittsburgh, Pa.), Fluka (Milwaukee, Wis.), and Sigma Chemical Co. (St. Louis, Mo.). Gases were purchased from (Northeast Airgas, Cheshire, Conn.).

**Bacterial cultures.** *Desulfovibrio desulfuricans* IC1 (= DSM 12129) was obtained from our collection. *Desulfitobacterium hafniense* was kindly provided by Jan Gerritse of the University of Groningen, Groningen, The Netherlands.

**Enrichment and cultivation of bacteria.** *Desulfovibrio desulfuricans* IC1 and strain T1 (see below) were maintained and grown in the mineral salts medium of Widdel and Pfennig (53); freshwater, saltwater, and brackish conditions were created by adjusting the NaCl and  $MgCl_2$  concentrations. Sulfate was omitted when cultures were grown with sulfonates. When organisms were tested for growth with taurine as the sole carbon, energy, and nitrogen source,  $NH_4Cl$  was omitted and the gas used was a mixture containing 75% (vol/vol) Ar and 25% (vol/vol)  $CO_2$ . Titanous chloride (the amount added was just sufficient to turn the redox indicator colorless) was used as a reductant when strain T1 was grown with nitrate as a TEA. Substrates were added from separately sterilized 0.5 to 1 M stock solutions.

Strain T1 was isolated from an enrichment culture by using sulfide-rich black marine mud obtained from a marsh (Marsh Gardens, East Coast Highway, Republic of Singapore). The primary liquid enrichment culture contained 10 mM malate and 10 mM taurine as the electron donor and the electron acceptor, respectively.

Solidified media in plastic petri dishes were used to obtain pure cultures. We

\* Corresponding author. Mailing address: Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269-2131. Phone: (860) 486-1931. Fax: (860) 486-1936. E-mail: erl@uconnvm.uconn.edu.

<sup>†</sup> Present address: Department of Microbiology, University of Washington, Seattle, WA 98195.

added 10 mM taurine as the sole carbon and energy source and agarose (1.5%, wt/vol) as a solidifying agent to the mineral salts medium. After the pH was adjusted to 7 to 7.4 and after autoclaving, the medium was cooled to ca. 80°C, and the mineral mixture, vitamins, and resazurin were added (52). The preparation was immediately transferred into an anaerobic hood, and then bicarbonate buffer and sodium sulfide reductant were added to final concentrations of 30 and 1.5 mM, respectively. The medium was then mixed, immediately poured into petri dishes, allowed to solidify, and then stored in Brewer type jars in the anaerobic chamber. Enrichment cultures were streaked onto the media outside the anaerobic hood, and the plates were then immediately returned to the hood for incubation in jars. The plates were incubated for 1 to 2 weeks before bacteria from single colonies were streaked onto new media.

Stock cultures were stored in liquid medium at 4°C and were transferred at 1- to 2-month intervals. A culture of isolate T1 has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, under accession no. DSM 12130.

**Analytical conditions.** Organic acids, hydrogen sulfide, and growth were detected and quantified as described previously (34). Taurine was quantified by the high-performance liquid chromatography method used to quantify organic acids; a linear response was observed for taurine concentrations ranging from 2.5 to 20 mM. Nitrate concentrations were measured by suppressed ion chromatography with conductivity detection by using an IonPac AS4A-SC analytical column. The eluant was 1.8 mM Na<sub>2</sub>CO<sub>3</sub>-1.7 mM NaHCO<sub>3</sub>, and the flow rate was 2 ml/min. Protein was quantified by a modified Lowry procedure (35). Poly- $\beta$ -hydroxybutyrate (PHB) was detected as crotonic acid and was quantified by using the assay of Law and Slepecky (31). Desulfoviridin was detected spectrophotometrically by determining the presence of a peak at 630 nm (26, 44, 53) and also by using the fluorescence test of Postgate (41). DNA G+C content and menaquinone analyses were performed by Hans Hippe of the DSMZ.

**Electron microscopy. (i) Transmission electron microscopy.** Bacteria in the late exponential or early stationary phase were used for transmission electron microscopy. Cells grown in a liquid culture were concentrated by centrifugation and gently resuspended in a smaller volume of culture medium. The resulting concentrate was then pipetted onto UV-irradiated (15 min) Formvar- and carbon-coated grids and allowed to settle for 1 to 2 min. The cells were negatively stained with phosphotungstate for 1 min. Excess stain was removed by blotting, and the preparation was viewed with a Philips model EM 300 electron microscope at an accelerating voltage of 80 kV.

**(ii) Scanning electron microscopy.** A few drops of a cell suspension in the mid-exponential to late exponential phase were placed onto cut silicon wafers (area, approximately 1 mm<sup>2</sup>) that had been coated with poly-L-lysine (0.1%, wt/vol). The cells were allowed to settle for 5 to 10 min. Attached cells were then fixed with a solution containing 1.5% (wt/vol) glutaraldehyde and 1.5% (wt/vol) formaldehyde in 0.1 M HEPES buffer (pH 7.6) containing 3 mM MgCl<sub>2</sub> for 1 h. After two washes in distilled water, the cells were postfixed with 1% (wt/vol) OsO<sub>4</sub> in distilled water overnight. Samples were washed three times in distilled water and then dehydrated twice in a graded series of ethanol solutions (50, 70, and 100% [vol/vol] ethanol). The wash solutions were partially drained in order to leave ca. 10% of the solution, so that the silicon wafers remained immersed. New solutions were added gently, so spinae were not detached from the cells. The wafers were then dried with a critical point dryer (Polaron model E3000) for 2 h, sputter coated (Polaron model E5100) with gold and palladium, and then viewed with a Zeiss model DSM 982 Gemini field emission scanning electron microscope operated at an accelerating voltage of 2 kV.

**Isolation of nucleic acids and sequencing.** One milliliter of a culture of strain T1 was placed in a sterile microcentrifuge tube and centrifuged for 10 min at 13,500  $\times$  g. The supernatant was discarded, and the pellet was resuspended in water. The contents were then vortexed and boiled for 7 min, which yielded a crude DNA template. Nearly full-length 16S ribosomal DNA (rDNA) was amplified in four sets of 100- $\mu$ l reaction mixtures containing 3  $\mu$ l of DNA template, 0.2  $\mu$ M universal primer fD1, 0.2  $\mu$ M universal primer rD1 (49), 200  $\mu$ M dATP, 200  $\mu$ M dCTP, 200  $\mu$ M dGTP, 200  $\mu$ M dTTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, and 3.75 U of AmpliTaq DNA polymerase (Perkin-Elmer Corp., Norwalk, Conn.).

The PCR was performed with a Perkin-Elmer model 2400 thermal cycler by using the following conditions: primary denaturation at 94°C for 2 min and 35 cycles consisting of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, and DNA extension at 72°C for 1 min. The reaction mixtures were kept at 72°C for 7 min, and then the PCR was terminated. The four PCR mixtures were combined. The presence of nearly full-length 16S rDNA was confirmed by horizontal agarose gel electrophoresis, which yielded a single band of the expected size. The PCR amplicon was then purified with a Qiagen (Chatsworth, Calif.) column and quantified with a model DyNA Quant 200 DNA fluorometer (Hoefer Pharmacia Biotech, Inc., San Francisco, Calif.).

The 16S rDNA amplicon was cycle sequenced and analyzed with an Applied Biosystems Prism sequencer (Perkin-Elmer). The initial sequence was generated with primers fD1 and rD1 by using an Applied Biosystems cycle sequencing kit. The entire amplicon was then cycle sequenced with primers spaced approximately 300 bases apart. The 16S rDNA amplicon was completely sequenced in both directions.

**Phylogenetic inference.** Searches performed with FASTA (39) and BLAST (54) revealed that the 16S rDNA gene of strain T1 was closely related to the 16S

rDNA genes of sulfate-reducing members of the  $\delta$  subclass of the class *Proteobacteria* ( $\delta$ -*Proteobacteria*). The GCG package (10) run on a VAX computer was used to retrieve the following 16S rDNA signatures: AB015241 of unidentified proteobacterial strain JTB20, L42613 of "*Desulfohopalus vacuolatus*," X99707 of *Desulfofustis glycolicus*, X95181 of *Desulfocapsa thiozymogenes*, M34411 of *Desulfobulbus* sp., X95180 of *Desulfobulbus elongatus*, M34410 of *Desulfobulbus propionicus*, L07834 of *Geobacter metallireducens*, X70954 of *Pellobacter propionicus*, M26634 of *Desulfuromonas acetoxidans*, X83274 of *Desulforhabdus arnigenus*, L27426 of *Desulfacinium infernum*, M34403 of "*Desulfoarcus baarsii*," X85131 of *Syntrophus buswellii*, X85132 of *Syntrophus gentianae*, X93994 of *Desulfovibrio* sp., and J01695 of *Escherichia coli*.

The sequences were aligned and edited by using ClustalW. Sites that did not contain at least 50% of one base were deleted from the alignment. Neighbor-joining and parsimony trees (Parsimony trees are not shown) were generated by using PHYLIP (18), and maximum-likelihood trees (data not shown) were generated by using PUZZLE (45). The trees were viewed in TREEVIEW (38). Phylogenetic trees were also constructed for an unedited alignment containing all sites.

**Cell extracts and enzymology.** Cells were grown in 500-ml bottles capped with screw caps with butyl rubber stoppers, which allowed us to introduce or withdraw substrates with syringes. The cells were harvested by centrifugation and washed with marine buffer (343 mM NaCl, 6 mM MgCl<sub>2</sub>, 10 mM Tris-HCl; pH 7.2) three times. After resuspension in a small volume of the buffer, the cells were broken with a French pressure cell at 15,000 lb/in<sup>2</sup>. The preparation was centrifuged at 10,000  $\times$  g, and the supernatant (cell extract) was used for enzyme assays. Adenylphosphosulfate (APS) reductase assays were performed as described previously (33).

**Nucleotide sequence accession number.** The 16S rRNA-encoding DNA sequence of strain T1 has been deposited in the GenBank database under accession no. AF118453.

## RESULTS

**Enrichment cultures.** In the primary enrichment culture containing malate plus taurine, growth and hydrogen sulfide were detected after about 1 week of incubation at 28°C. The enrichment culture consisted predominantly of vibrios and some rods. A secondary enrichment culture containing taurine (10 mM) as the sole carbon and energy source contained predominantly rod-shaped bacteria after about 1 week; millimolar concentrations of hydrogen sulfide were detected. A pure culture of strain T1 was obtained from the taurine enrichment culture after five sequential streaking procedures to obtain well-separated colonies. Culture purity was confirmed microscopically and by the lack of aerobic or anaerobic growth in complex medium (AC medium [Difco]) under marine and nonmarine conditions.

**Morphology and physical characteristics.** Cells of strain T1 were rod shaped; the cell lengths ranged from 1.7 to 2.3  $\mu$ m, and the cell widths ranged from 0.9 to 1.2  $\mu$ m. Often the cells were in chains containing up to six cells. As determined by electron microscopy, some cells contained nonprosthecae structures called spinae (14, 15) (Fig. 1 and 2). The spinae that were produced appeared to be the type of spinae produced by marine pseudomonad strain D7 (13). The bases of the spinae were flared, and the spinae appeared to be hollow from the base to the tip (Fig. 1, arrow). The spinae appeared to be somewhat flexible, and some of the spinae were as long as 2.5  $\mu$ m. Spinae were observed on cells grown by anaerobic respiration with malate plus sulfate, as well as on cells fermenting taurine or pyruvate, although we noticed more spinae on cells grown with taurine; no detailed studies were made to determine which conditions resulted in maximum spinae production. Cells were not motile, nor were flagella detected by electron microscopy. Spores were not observed, and no growth was detected after pasteurization at 80°C for 10 min; *Desulfotribacterium hafniense* (which forms endospores) was the positive control used.

**Growth conditions and physiological characteristics.** The growth temperature range tested was 17 to 37°C. We found that strain T1 is a mesophile with an optimum growth temperature of about 31°C, and growth occurred at temperatures of

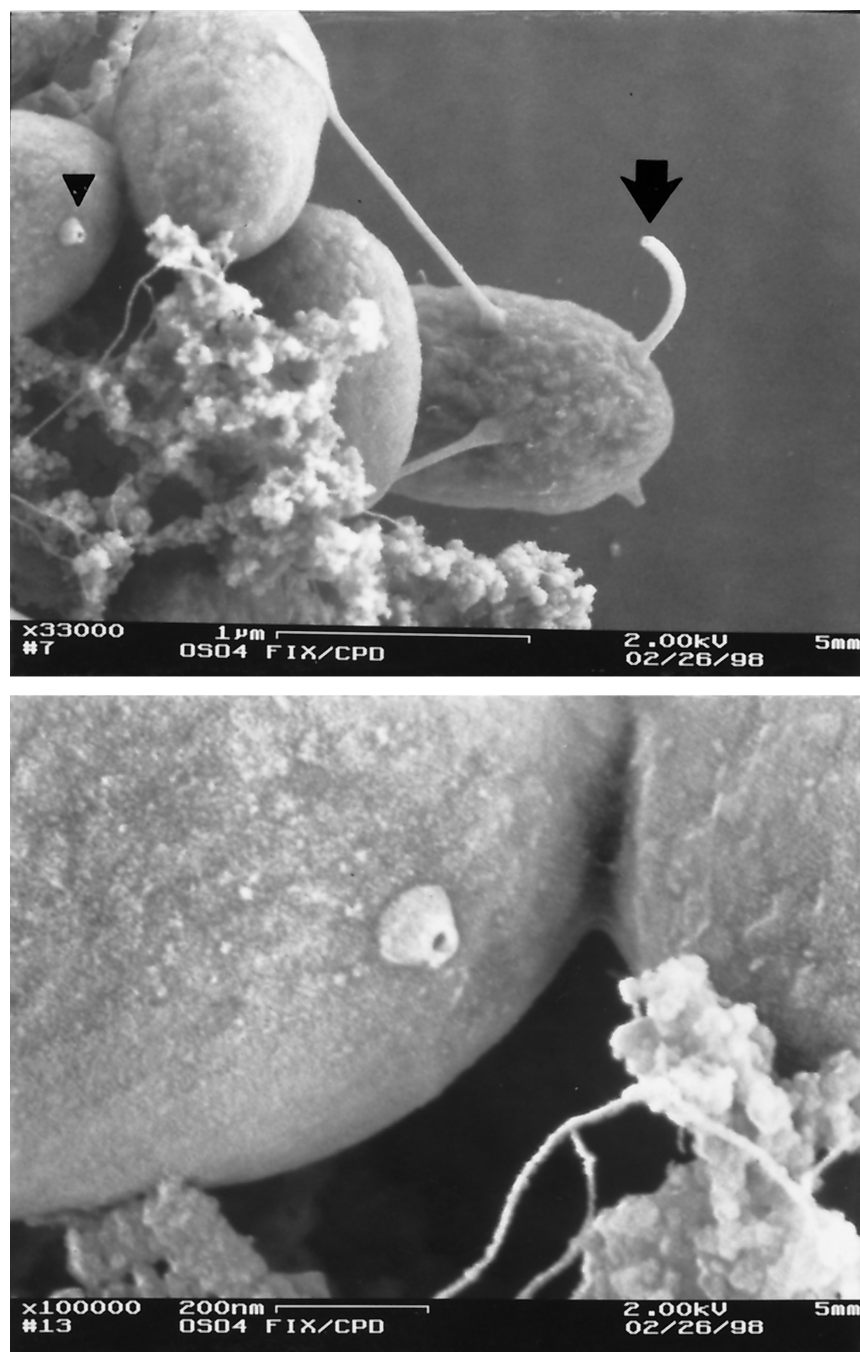


FIG. 1. (a) Scanning electron micrograph of strain T1 grown with taurine as the sole carbon and energy source. The arrow indicates a spina; the arrowhead indicates a truncated spina. Bar = 1  $\mu$ m. (b) magnified image of the truncated spina in panel A. Bar = 200 nm.

20 to 35°C. No growth occurred at 17 or 37°C. Growth occurred at pH 6.0 to 8.2, and the optimum pH was 7.4. Growth occurred in brackish medium but not in freshwater medium. The electron donors that supported growth with sulfate included formate (40 mM), ethanol (5 mM), propanol (10 mM), butanol (10 mM), lactate (10 mM), pyruvate (10 mM), propionate (10 mM), malate (10 mM), fumarate (10 mM), succinate (10 mM), Casamino Acids (0.2%, wt/vol), butyrate (5 mM), isobutyrate (5 mM), and alanine (10 mM). The electron donors tested that did not support growth included acetone (10 mM),

acetate (10 mM), glycolate (5 mM), glyoxylate (5 mM), ethylene glycol (5 mM), tetraethylene glycol (5 mM), ethylamine (5 mM), ethanolamine (5 mM), cysteine (10 mM), glucose (10 mM), and benzoate (2.5 mM). The organic substrates that supported fermentative growth included taurine (5 mM) and pyruvate (10 mM). Growth occurred with sulfite (10 mM) as the sole source of energy when 2.5 mM acetate was a source of carbon; no growth occurred when thiosulfate (20 mM) replaced sulfite. Fumarate, lactate, succinate, malate, isethionate, cysteate, coenzyme M, bromoethanesulfonate, sulfosuccinate, and



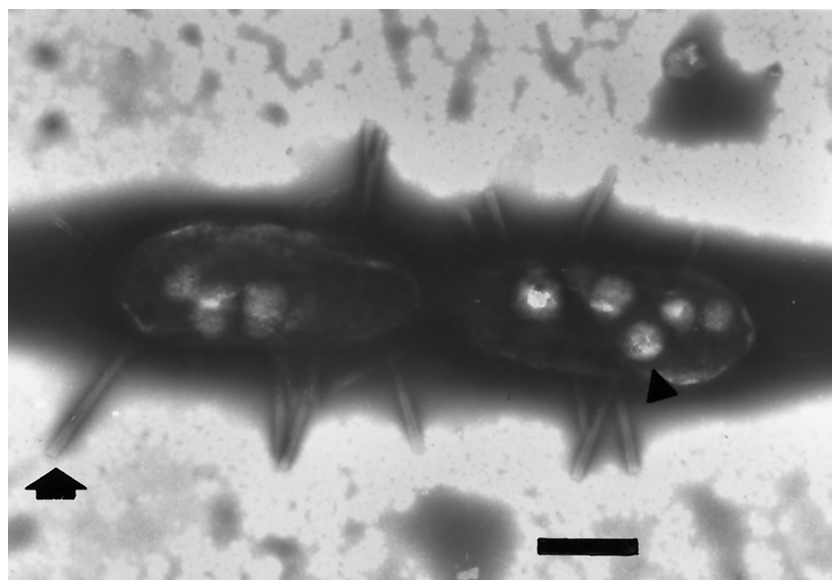


FIG. 2. Transmission electron micrograph of strain T1 grown with taurine as the sole carbon and energy source. Cells were negatively stained with phosphotungstate. The arrow indicates a representative spina; the arrowhead indicates an inclusion body. Bar = 2  $\mu$ m.

3-aminoethanesulfonate (each at a concentration of 10 mM) were not fermented. When malate was the carbon and energy source, the TEA that supported growth included sulfite (5 mM), thiosulfate (10 mM), sulfate (10 mM), and nitrate (10 mM). However, when sulfide (final concentration, 0.5 mM) was used as a reductant instead of titanous chloride, strain T1 did not grow with nitrate as the TEA. The TEA that did not support growth included dimethyl sulfoxide (10 mM), fumarate (10 mM), and the sulfonates isethionate (10 mM), cysteate (10 mM), coenzyme M (10 mM), bromoethanesulfonate (10 mM), and sulfosuccinate (10 mM).

Desulfovirodin was not detected in cell extracts; cell extracts of sulfate-grown strain IC1 cells were used as the positive control. The cells contained menaquinone 5 ( $H_2$ ), as revealed by high-performance liquid chromatography. The G+C content of the DNA was  $50.6 \pm 0.2$  mol%.

**Growth with taurine as a fermentable substrate and production of inclusion bodies.** Taurine served as a sole source of carbon, energy, and nitrogen for growth when it was used in the absence of ammonia and dinitrogen. The soluble end products obtained from taurine fermentation were ammonia, acetate, and hydrogen sulfide. At taurine concentrations of approximately 11 and 16 mM, about 83% of the carbon was

recovered (Table 1). Cells grown with taurine also contained large numbers of inclusion bodies (Fig. 2), which were found to be PHB. Taurine-grown cells and cells grown on malate plus sulfate contained 50 mg of PHB per g (wet weight) (25% of the cell dry weight) and 14 mg of PHB per g (wet weight) (7% of the cell dry weight), respectively.

The sulfur of taurine was quantitatively recovered as hydrogen sulfide, and most of the nitrogen of taurine was recovered as ammonia when the initial concentrations of taurine were ca. 5 and 11 mM and taurine was completely utilized (Table 1). At a taurine concentration of ca. 16 mM, about 7% of the taurine remained; no further increase in acetate production was observed. Sulfide at concentrations of about 15 mM appeared to inhibit growth.

When cells were grown with ca. 11 mM taurine and 10 mM nitrate as the electron donor and the electron acceptor respectively, nitrate was not utilized as a TEA (Table 1). The taurine, however, was completely consumed; sulfide, ammonia and acetate were detected at concentrations essentially identical to the concentrations obtained when approximately the same concentration of taurine was the sole carbon, energy, and nitrogen source in the absence of added nitrate (Table 1).

TABLE 1. Stoichiometric relationship between substrates utilized and end products detected

Substrate(s) provided	Substrate remaining	Acetate		Sulfide <sup>c</sup>		Ammonia <sup>c</sup>	
		Concn detected (mM)	% of theoretical value <sup>b</sup>	Concn detected (mM)	% of theoretical value <sup>b</sup>	Concn detected (mM)	% of theoretical value <sup>b</sup>
Taurine (5.42 mM)	ND <sup>d</sup>	1.61	59	5.44	100	3.8	70
Taurine (11.33 mM)	ND	4.73	83	11.58	102	11	95
Taurine (16.33 mM)	Taurine (1.19 mM)	6.22	82	14.26	94	14	98
Taurine (11.63 mM) <sup>a</sup>	ND	3.79	65	12.16	105	9.9	81
Taurine (11.43 mM) + nitrate (10 mM) <sup>a</sup>	Nitrate (10 mM)	3.19	56	12.64	111	11.43	90

<sup>a</sup> Titanous chloride replaced sulfide as the reductant.

<sup>b</sup> Percentages were calculated based on the following equation (see text):  $C_2H_7O_3NS \rightarrow 0.5 C_2H_4O_2 + CO_2 + NH_4^+ + HS^-$ .

<sup>c</sup> Values were corrected for the initial sulfide concentration and the initial ammonia concentration used as the reductant and the nitrogen source, respectively.

<sup>d</sup> ND, not detected.

TABLE 2. Stoichiometry of substrates utilized and end products formed<sup>a</sup>

Carbon source utilized	Optical density at 650 nm	Acetate concn (mM)	Sulfide concn (mM)	Ratio of carbon source utilized to acetate produced to sulfide produced
Lactate (4.8 mM)	0.17	3.5	3.6	1:0.73:0.75
Fumarate (4.9 mM)	0.40	0	5.7	1:0:1.16

<sup>a</sup> The initial sulfate concentration was 10 mM.

**Stoichiometry of substrate conversion coupled to sulfate reduction.** Growth with fumarate (4.9 mM) plus sulfate (10 mM) resulted in production of sulfide (5.7 mM), but acetate was not detected. In cultures containing lactate (4.8 mM) plus sulfate (10 mM), sulfide (3.6 mM) and acetate (3.5 mM) were detected as end products (Table 2). The final optical densities at 650 nm after growth with lactate and after growth with fumarate were 0.17 and 0.40, respectively.

**APS reductase levels during growth with various substrates and effects of sulfate analogs on growth with taurine.** Cells grown with sulfate as the TEA had a APS reductase specific activity of 0.83  $\mu\text{mol}$  of ferricyanide reduced/min/mg of protein, while taurine-fermenting cells had a lower specific activity (0.31  $\mu\text{mol}$  of ferricyanide reduced/min/mg of protein). Molybdate at a concentration of 5 mM and 10 mM tungstate inhibited strain T1 growth with 10 mM taurine; no growth was observed when the culture was incubated for 1 month.

**Phylogenetic characteristics.** The phylogenetic trees generated by the maximum-likelihood, neighbor-joining, and parsimony algorithms were in virtual agreement for both edited and nonedited alignments. Agreement of phylogenetic trees for members of the  $\delta$ -*Proteobacteria* when edited or nonedited alignments were used has been described previously (20). All of the trees placed strain T1 close to unidentified strain JTB20 and "*Desulforhopalus vacuolatus*" (23). *Desulfofustis glycolicus* (20) also branched close to strain T1. The maximum-likelihood tree (data not shown) suggested that "*Desulforhopalus vacuolatus*," strain T1, and strain JTB20 had a common ancestor. The neighbor-joining tree in Fig. 3 shows that the bootstrap value for separation of *Desulfofustis glycolicus* from "*Desulforhopalus vacuolatus*," strain T1, and JTB20 was 99%. The levels of similarity between strain T1 and JTB20, "*Desulforhopalus vacuolatus*," and *Desulfofustis glycolicus* were 95.3, 93.0, and 91.5%, respectively. Additional phylogenetic support for clustering "*Desulforhopalus vacuolatus*" with strain T1 and JTB20 includes the presence of a unique 13-base deletion found in the unedited 16S rDNA alignment for only these three organisms. The 16S rDNA of *Desulfofustis glycolicus* does not contain this deletion. Many of the members of the  $\delta$ -*Proteobacteria* in our alignment have an approximately 18-base insertion between *E. coli* nucleotide positions 186 and 187. The 13-base deletion is found in this insertion region. Because this stretch of 16S rDNA was found to be hypervariable, these sites were not used in the alignments used to produce Fig. 3. However, we consider the deletion significant and believe that it may be a phylogenetic characteristic of species belonging to the genus "*Desulforhopalus*."

## DISCUSSION

**Taurine fermentation.** The detected products of taurine fermentation by strain T1 were acetate, ammonia, and sulfide. Based on measurements of the substrates utilized and the products detected, we believe that the fermentation is repre-

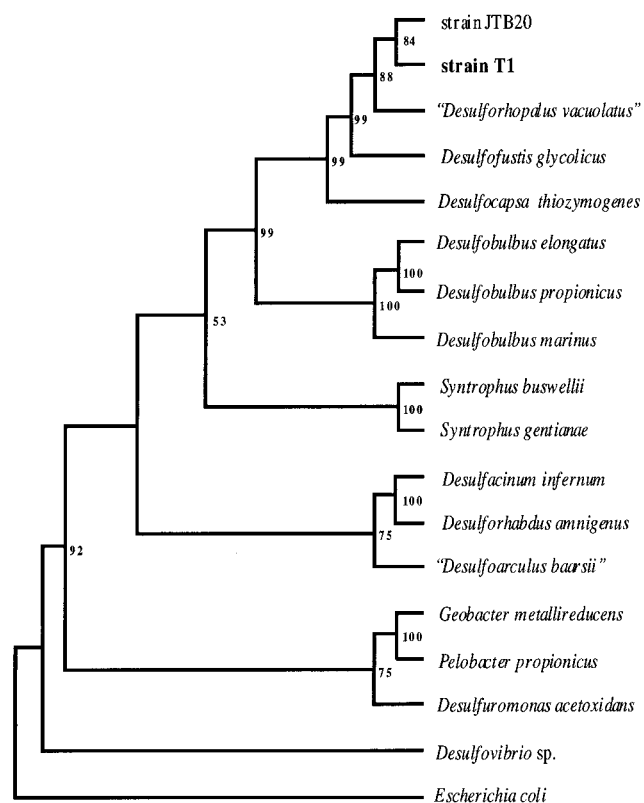


FIG. 3. Neighbor-joining cladogram based on  $\delta$ -proteobacterial 16S rDNA sequences. Sequences were aligned and edited in ClustalW. Hypervariable sites (as determined by less than 50% consensus of one base per alignment site) were removed. The aligned sequences were analyzed in PHYLIP. Seqboot was used to generate 500 bootstrap data sets. The bootstrap data sets were used to produce 500 distance matrices in DNADIST. The Kimura two-parameter model was used in DNADIST to account for probable variance in base substitution rates. Transversions were weighted twice as heavily as transitions were weighted. Neighbor-joining trees were constructed from the matrices by using NEIGHBOR. A consensus tree was generated in CONSENSE. The consensus tree was rooted with *E. coli* and was viewed in TREEVIEW. The numbers are bootstrap percentages. The levels of similarity between strain T1 and JTB20, "*Desulforhopalus vacuolatus*," and *Desulfofustis glycolicus* were 95.3%, 93.0, and 91.5%, respectively.

sented by the following equation:  $\text{C}_2\text{H}_7\text{O}_3\text{NS} \rightarrow 0.5 \text{C}_2\text{H}_4\text{O}_2 + \text{CO}_2 + \text{NH}_4^+ + \text{HS}^-$ , where  $\Delta G'^{\circ} = -136.99$  kJ/mol of taurine (46). This contrasts with taurine fermentation in another obligate anaerobe, in which the sulfurous end product was thiosulfate, not sulfide (9). Whether taurine fermentation in strain T1 proceeds by the initial reactions demonstrated for the taurine-fermenting syntrophomonad (9) remains to be determined. The proposal of Cook (6) that transamination of taurine to sulfoacetaldehyde is followed by hydrolytic release of sulfite from sulfoacetaldehyde and that this process occurs during taurine metabolism in both aerobes and anaerobes, either for assimilatory or dissimilatory purposes, is an attractive proposal and is consistent with the overall stoichiometry proposed above.

PHB was produced in cells of strain T1 during taurine fermentation, as well as when malate oxidation was coupled to sulfate reduction. The amount of PHB produced during taurine fermentation was approximately three times more than the amount of PHB produced by cells grown on malate plus sulfate per gram (wet weight) of cells. A more detailed study of

PHB production as it relates to taurine carbon assimilation is warranted.

Taurine-grown cells had an APS reductase specific activity that was less than one-half the APS reductase specific activity of sulfate-grown cells, suggesting that the sulfur of taurine is not released as sulfate and then assimilated. Low APS reductase activity was also exhibited by SRB utilizing TEA other than sulfate, including isethionate (33) or nitrate (12), for growth. Despite the fact that taurine-grown cells had a reduced level of APS reductase activity (and presumably ATP sulfurylase activity as well), it was surprising that this organism failed to grow with taurine in the presence of either molybdate or tungstate (which are considered competitive inhibitors of ATP sulfurylase and inhibit growth by effecting degradation of intracellular ATP [37]). It seems probable that the decreased ATP sulfurylase activity is nonetheless sufficient to deplete the ATP in cells.

#### Growth with taurine under nitrate-reducing conditions.

When sulfide (ca. 0.5 mM) was used as a reductant, strain T1 did not grow with malate plus nitrate; when titanous chloride was used as a reductant, growth via nitrate reduction to ammonia occurred. The apparent inhibition by sulfide is consistent with the sulfide inhibition of nitrate ammonification reported for a freshwater *Desulfovibrio* isolate (7).

Since strain T1 could ferment taurine but also reduced nitrate, we attempted to grow this isolate with taurine and nitrate as the electron donor and the electron acceptor, respectively (these conditions supported growth of a strain of *Acaligenes* sp. [8]). Titanous chloride was used as a reductant, and the inoculum had previously been grown with nitrate so as to not carry over any contaminating sulfide. We were surprised to observe that coupling of taurine oxidation to nitrate reduction did not occur and that instead taurine was fermented preferentially. Whether the sulfur of taurine is released as sulfide, thereby inhibiting the ability to grow with nitrate, or whether the sulfur is released as sulfite, which is then reduced in preference to nitrate, remains to be studied. *Desulfovibrio desulfuricans* Essex 6 (43) reduced nitrate in preference to sulfate when both TEA were present; however, other *Desulfovibrio* spp. could reduce nitrate and sulfate simultaneously (24, 36). Clearly, regulation of the metabolism of nitrate and sulfur compounds needs further study.

**Oxidation of substrates during sulfate reduction.** The fact that acetate accumulated during lactate oxidation and fermentation of taurine by strain T1 suggests that this isolate should be considered an incomplete oxidizer; the lack of acetate accumulation when fumarate was oxidized indicates otherwise. Other than *Desulfobacter* spp., SRB that are considered complete oxidizers are known to excrete acetate, or not to excrete acetate, depending on the electron donor respired (51); one such SRB had (19) a ratio of amount of lactate consumed to amount of acetate (and sulfide) accumulated similar to the ratio obtained for strain T1.

**Spinae.** Spinae production is not limited to a particular physiological group (13), and thus far spinae have been found in *Pseudomonas* spp. (15), a *Chlorobium* sp. (4, 5), and a *Synechococcus* sp. (40). This apparently is the first report of spinae production by a SRB. Whether the spinae in this SRB play roles that have been proposed for spinae in other bacteria (2, 13) remains to be determined.

**Ecological significance.** Significant concentrations of sulfonates occur in the environment; these compounds account for 20 to 40% of the total organic sulfur in marine sediments (48) and at least 50% of the total organic sulfur in a variety of forest soils (1). This report and other reports of sulfidogenesis from anaerobic sulfonate dissimilation (29, 30, 33, 34) underscore

the potential for sulfonates to be significant sources of hydrogen sulfide, as well as carbon sources, in various habitats. Sulfonate sulfur reduction may thus account for reported sulfide concentrations that are significantly higher than the concentrations expected based on the pool of sulfate present (11, 17). Since taurine fermentation by members of two physiologically distinct bacterial groups leads to production of different sulfurous end products (thiosulfate [9] and the bisulfide ion [this study]), important implications for the participation of sulfonate sulfur in the global sulfur cycle are evident.

**Phylogenetic position.** The results of the phylogenetic analyses, as well as the presence of a unique 13-base deletion in both "*Desulforhopalus vacuolatus*" and strain T1, indicate strongly that strain T1 should be considered a member of the genus "*Desulforhopalus*." The fact that a third uncharacterized bacterium (strain JTB20) may also fall into this genus is interesting; unfortunately, no information concerning this bacterium's physiology is available.

**Description of *Desulforhopalus singaporensis* sp. nov.** *Desulforhopalus singaporensis* (sin.ga.po'rensis. M.L. n. *Singapore*, Republic of Singapore; L. suff. *-ensis*, native of; M.L. adj. *Singaporensis*, native of Singapore, referring to the place of isolation). Gram-negative cells that are 1.7 to 2.3  $\mu\text{m}$  long and 0.9 to 1.2  $\mu\text{m}$  wide. Endospores not detected. Cells may grow as chains containing two to six cells. No flagella. Cells able to produce the nonprosthecae structures called spinae. Strict anaerobe. Grows with 2-aminoethanesulfonate (taurine) as a sole carbon, energy, and nitrogen source without an additional TEA. Grows with sulfite as the sole source of energy with acetate as the source of assimilatory carbon. Grows chemotrophically with oxidation of formate, lactate, pyruvate, fumarate, succinate, butyrate, ethanol, butanol, or Casamino Acids coupled to reduction of sulfate; slow growth occurs with propionate, isobutyrate, and caproate. Acetate accumulates from lactate oxidation but not from fumarate oxidation.  $\text{H}_2$  and acetate are not utilized. Pyruvate is fermented. Sulfite, thiosulfate, and nitrate serve as alternate TEA for growth; 0.5 mM sulfide inhibits growth on nitrate. Desulfovirdin not detected. Cells contain menaquinone 5( $\text{H}_2$ ). The pH range for growth is 6.0 to 8.2, and the optimum pH is 7.4. The temperature range for growth is 20 to 35°C, and the optimum temperature is 31°C. Isolated from marine sulfidogenic mud from a saltwater marsh in the Republic of Singapore. The G+C content of the genomic DNA is 50.6  $\pm$  0.2 mol%. The type strain is S'pore T1, which has been deposited in the DSMZ as strain DSM 12130.

#### ACKNOWLEDGMENTS

We thank Terry Beveridge of the University of Guelph, Guelph, Ontario, Canada, for comments on bacterial spinae and David Benson and Jeffrey Gawronski for helpful advice and discussions about the phylogeny of strain T1. We thank Marie Cantino, Lamia Khairallah, Steve Daniels, and Jim Romanow of the Electron Microscopy Facility (University of Connecticut) for the excellent electron microscans. We appreciate helpful discussions with Pieter Visscher, the use of his ion chromatograph, and the technical assistance of Shelley Hoef and Dan Rogers. Valuable comments from anonymous reviewers improved the manuscript.

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