Sulfate Reduction by a *Desulfovibrio* Species Isolated from Sheep Rumen

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Received for publication 23 January 1974

Several dissimilatory, sulfate-reducing bacteria were isolated from the rumen fluid of sheep fed purified diets containing sulfate. One isolate, strain D, was selected for characterization. This organism is a nonsporeforming, obligately anaerobic, mesophilic, nonmotile, gram-negative, straight rod. Cell-free extracts show absorption maxima for cytochrome c₅₅ and desulfoviridin, characteristic of *Desulfovibrio*. Carbohydrates, as a sole carbon source, will support growth. Lactate supports growth in the presence of sulfate, not in its absence, whereas glucose or pyruvate support growth either in the presence or absence of sulfate. The isolate has a deoxyribonucleic acid base composition of 61.2% guanine plus cytosine, which is similar to that of several other species of *Desulfovibrio*; however, it differs from previously described species in morphology, motility, and carbon source utilization. Cell-free extracts of this bacterium exhibit adenosine 5'-triphosphate-sulfurylase, adenosine-5'-phosphosulfate-reductase, and hydrogenase activity. After incubation of cell-free extracts with adenine 5'-triphosphate and *32SO₄²⁻*, adenosine-5'-phosphosulfate rather than 3'-phospho-adenosine-5'-phosphosulfate was shown to be labeled, indicating that the pathway of sulfate reduction in this organism is similar to that of other dissimilatory sulfate reducers. This is the first report of a *Desulfovibrio* sp. isolated from the rumen.

Sulfate reduction is known to occur in ruminants (3, 18). Although Lewis (18) proposed that a *Desulfovibrio*-like organism might be responsible for this sulfate reduction, he was unable to isolate such an organism. By preheating rumen fluid which contained only about 10⁴ sulfate-reducing organisms per ml, Coleman (8) was able to isolate a sulfate-reducing bacterium from hay-fed sheep dosed with sulfate. This organism was simply referred to as Coleman’s organism for a number of years (5, 29), until the dissimilatory sulfate-reducing bacteria were reclassified into the two genera *Desulfotomaculum* (7) and *Desulfovibrio* (30). At that time, Coleman’s organism was reclassified as *Desulfotomaculum ruminis*, since among other characteristics it is sporeforming and contains a b-type cytochrome. Coleman (8) has suggested that the low numbers of *D. ruminis* found in the sheep rumen would indicate that this organism is not a significant sulfate-reducing bacterium in ruminants. However, Mara and Williams (20) report that there is no media available yet which will give reliable viable counts of *Desulfotomaculum* sp.

We have shown previously that washed rumen microorganisms, from sheep fed a relatively high concentration of sodium sulfate as their only dietary source of sulfur, exhibit a high rate of sulfate reduction which is inhibited by molybdate (12). Molybdate is also known to inhibit sulfate reduction in *Desulfovibrio desulfuricans* (25) and the other anaerobic sulfate-reducing bacteria which have been shown to contain adenosine 5'-triphosphate (ATP)-sulfurylase (ATP:sulfate adenylyltransferase, EC 2.7.7.4) (1). To clarify the role of sulfate in the copper-molybdenum-sulfate interaction observed in ruminants (13), a study was begun to isolate sulfate-reducing bacteria from the rumen of sheep fed sulfate-containing purified diets. This paper describes the isolation, characterization, and preliminary studies of the pathway of sulfate reduction of bacteria which exhibits a high rate of sulfate reduction to sulfide.

MATERIALS AND METHODS

Source of rumen fluid. Rumen fluid was obtained

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¹ Paper no. 4235 of the Journal series of the North Carolina State University Agricultural Experiment Station.
from sheep fitted with a rumen fistula and fed a modification of the sulfur adequate purified non-protein diet of Whanger and Matrone (30). This diet contains 1.2% sodium sulfate as the sole dietary source of sulfur. Albert et al. (2) reported this level of sodium sulfate as the requirement for lambs fed a purified ration with urea as the nitrogen source. Other ingredients in the diet include 4.6% urea, 35.8% glucose, 32.4% starch, 4.0% fat, 3.0% cellulose, 4.0% KHCO₃, 6.0% NaHCO₃, 1.0% CaHPO₄, 3.0% mineral mix (33), and 5.0% vitamin mix (22). Three animals were used in this study for both the isolation and determination of numbers of sulfate-reducing organisms present in the rumen fluid. The animals used had been maintained on this diet for a year before this study.

**Media.** The anaerobic sulfate-reducing bacteria were isolated in roll tubes and maintained in deep stab's of medium B of Butlin et al. (6), modified by the addition of 0.1% yeast extract, 10% clarified rumen fluid, 0.4% Na₂CO₃, 0.01% sodium thioglycollate, 10⁻¹% resazurin, and 1.5% agar, pH 6.8. The clarified rumen fluid was prepared by filtering crude rumen fluid, obtained from the animals described above, through cheesecloth, centrifuging at 4,000 x g for 30 min, boiling the supernatant for 10 min, and recentrifuging at 30,000 x g for 30 min.

For the initial growth of colonies picked from roll tubes, a modification of medium C of Pankhurst (24) was used. It contained the following: K₂HPO₄ (0.5 g); NH₄Cl (1.0 g); CaCl₂·2H₂O (0.1 g); MgSO₄·7H₂O (0.06 g); (NH₄)₂SO₄ (7.0 g); sodium lactate (60%) (5.0 g); clarified rumen fluid (100 ml); resazurin (0.001 g); Na₂S (0.08 g); and distilled water (1 liter), pH 6.8.

For general use and particularly for the biochemical studies, medium C of Butlin et al. (6) was used by modifying the sodium lactate (60%) to 5.0 g/liter and the FeSO₄·7H₂O to 0.004 g/liter and by adding cysteine hydrochloride (0.5 g/liter); NaHCO₃ (4.0 g/liter), and resazurin (0.001 g/liter), pH 6.8. This medium minus lactate, iron, and bicarbonate was used as a diluent.

For the determination of carbon source utilization, the procedures and media described by Postgate and Campbell (30) were used with the exception that NaHCO₃ (4 g/liter) was included in all of the media and the pH was adjusted to 6.8.

All media were prepared and dispensed anaerobically in the presence of oxygen-free 95% CO₂-5% H₂ by the techniques of Hungate (14) designed for studies with strictly anaerobic rumen organisms. Ferrous salts, NaHCO₃, Na₂CO₃, cysteine, and carbohydrates were sterilized by membrane filtration. The state of reduction was monitored by including the redox indicator, resazurin, in the medium (17).

**Growth and storage.** A 5 to 10% inoculum was used, since this amount yielded log-phase cultures in 20 to 24 h. A 1% inoculum required 40 h to reach the log phase. When large amounts of cells were required, cultures were grown in 3-liter round-bottom flasks. For general use, the liquid medium (10 ml) was kept under the gas mixture described above by means of rubber stoppers in 18-mm test tubes. Subcultures were made every 24 h. Except for the temperature studies, all cultures were incubated at 37 C.

Cultures were stored successfully either by refrigeration, freezing, or lyophilization. Cultures were stored for several weeks in liquid medium B or C in the refrigerator at 4 C. Deep stab's were made of medium A and stored at 0, -20, and -60 C for several months. Lyophilization with a Virtis freeze dryer was done by using standard procedures, except that all manipulations were carried out anaerobically.

**DNA base composition.** Deoxribonucleic acid (DNA) was isolated by the method of Marmur (21) as modified by Saunders et al. (33). Buoyant density was determined by cesium chloride density gradient centrifugation as described by Saunders et al. (33), except that *Escherichia coli* (p = 1.713 g/cm³) and *Micrococcus luteus* (p = 1.731 g/cm³) were used as reference standards. Buoyant density and base composition were calculated by the equations of Sueoka (34) to facilitate comparison with the base composition of other sulfate-reducing bacteria used in Postgate and Campbell’s classification (30).

**Preparation of cell-free extracts.** Cells were harvested by centrifugation at 4,000 x g, washed with 0.2 M 2-(N-morpholinol)-ethanesulfonic acid (MES) buffer (pH 6.8), diluted 1:1 with buffer, and passed once through the French pressure cell. Cell debris was removed by centrifugation at 30,000 x g for 30 min.

**Enzyme assays.** ATP-sulfurylase was assayed by the molybdic method of Wilson and Bandurski (37). The complete reaction mixture contained ATP (5 μmol); MgCl₂ (5 μmol); Na₂MoO₄ (10 μmol); MES buffer (pH 6.8, 50 μmol); purified yeast inorganic pyrophosphatase (50 μg); and cell-free extract (0.2 to 0.5 mg of protein) in a total of 0.75 ml. The control reaction mixture consisted of the complete reaction mixture minus the Na₂MoO₄. Incubation temperature was 37 C. The reaction was stopped by the addition of 0.4 ml of 10 N H₂SO₄ after 5, 15, and 30 min. Inorganic phosphate was determined by the method of Fiske and Subbarow (10) as modified by Gormori (11). Pyrophosphatase was obtained from Worthington. Since the crude cell-free extract did contain some adenosine triphosphatase activity, the most molybdate-dependent ATP sulfurylase activity was determined by subtracting the control (minus molybdate) activity from the complete reaction mixture activity.

Adenosine-5'-phosphosulfate (APS) reductase was assayed by the adenosine 5'-monophosphatase (AMP)-dependent reduction of ferricyanide in the presence of sulfitre as described by Peck et al. (27). The complete reaction mixture contained Na₂Fe(CN)₆ (4 μmol); AMP (10 μmol); Na₂SO₄ (10 μmol in 5 mM ethylenediaminetetraacetic acid); MES buffer (pH 6.8, 300 μmol); and cell-free extract (0.1 to 0.3 mg of protein) in a total of 3.0 ml. Incubation temperature was 37 C. The reaction was initiated by the addition of the sulfitre-ethylenediaminetetraacetic acid solution, and the initial rate of ferricyanide reduction was measured for 5 min at 420 nm by using a Unicam SP.800 B spectrophotometer. Since ferricyanide is also reduced to a lesser extent non-enzymatically, one control consisted of the complete reaction mixture minus the cell-free extract. The other control consisted of the complete reaction mixture minus AMP. The APS-reductase activity is reported as the net
activity dependent on sulfite, AMP, and cell-free extract.

Protein was determined by the method of Lowry et al. (19). All samples were run in duplicate for several different concentrations of cell-free extract. Both enzyme activities increased linearly with the level of protein and time.

Separation and identification of sulfur-containing nucleotides. Cell-free extracts were incubated with ATP and $^{35}$S$^{2-}$ as described by Peck (26) and modified by the use of MES buffer (pH 6.8). The complete reaction mixture contained: MES buffer (pH 6.8, 300 μmol: (MgCl$_2$ 20 μmol); ATP (20 μmol); Na$_2$SO$_4$, (2.5 μmol [containing 0.1 μCi of $^{35}$S-labeled sodium sulfate]); where indicated, Na$_2$MoO$_4$, (2 μmol); cell-free extract (5.0 mg of protein); total volume, 1.6 ml. Reaction mixtures were incubated for 15 min at 37 C, and the reaction was stopped by being placed in a boiling water bath for 3 min. The nucleotides were adsorbed on charcoal and eluted with 50% ethanol containing 0.01 N ammonium. The sulfur-containing nucleotides labeled with $^{35}$S were separated by paper electrophoresis by using the buffer described by Robbins and Lipmann (32), 0.03 M citrate (pH 5.9) with Whatman no. 1 paper, at 2 C for 1.5 h at 2,000 v (40 v/cm). Ultraviolet light was used to detect the nucleotides, which were cut out and counted in a gas-flow Nuclear Chicago Geiger counter. APS, purchased from Sigma Chemical Co., was used as a standard. The main contaminant in this preparation of APS was found to be AMP. $^{35}$S-labeled sodium sulfate was obtained from New England Nuclear Corp.

Rate of sulfate reduction. The procedure we have previously described (12) to measure the total amount of sulfate reduced in washed rumen microorganisms in a specified time was modified to allow the measurement of initial rates of sulfate reduction. A detailed description of this improved method follows.

Cells from a log-phase culture were washed anaerobically with 0.1 M phosphate buffer (pH 6.8) containing 0.01% sodium thioglycolate and suspended in the same buffer so that the diluted cells contained approximately 1 mg of protein per ml. Ten milliliters of the diluted cells was preincubated for 1 h, as suggested by Ishimoto et al. (15), in the presence of 50 μmol of sodium lactate and a continuous flow of oxygen-free 95% CO$_2$-5% H$_2$. The preincubation and reaction was done in a 25-ml Erlenmeyer flask connected to two other flasks in series by means of tygon tubing. The reaction flask was also attached to the gas source. Two zinc acetate-impregnated filter paper accordions (for trapping H$_2$S) were placed in the inlet and outlet tubes of the second flask, and a saturated solution of cadmium acetate was placed in the third flask of the series to monitor any carry-over of hydrogen sulfide as indicated by the formation of yellow cadmium sulfide.

After preincubation of the washed cells with lactate, the reaction was initiated by the addition of 30 μmol of sodium sulfate containing 0.5 μCi of $[^{35}]S$Na$_2$SO$_4$, bringing the total volume of the reaction mixture to 11 ml. The zinc-impregnated papers were replaced at 5-min intervals for 40 min and counted as described before (12). A portion of the washed, diluted cells was disrupted with a Branson sonifier, and protein was determined by the method of Lowry et al. (19). The rate of sulfate reduction is reported as micromoles of sulfide formed per hour per milligram of protein.

RESULTS

Isolation. Rumen fluid was serially diluted, and roll tubes were inoculated according to standard procedures used in the isolation of rumen microorganisms (14). The roll tubes contained the modified medium B of Butlin et al. (6). Since the sulfide-producing organisms form black colonies in roll tubes containing iron, these colonies were differentiated and counted to determine the number of viable sulfate-reducing bacteria per milliliter. Duplicate roll tubes made from rumen samples from each of three sheep on two separate days showed an average of 2.1 (±1.8) × 10$^7$ viable sulfate-reducing bacteria per ml of rumen fluid.

Individual black colonies from these roll tubes were transferred initially to the modified medium C of Pankhurst (24) and subsequently to modified medium C of Butlin et al. (6). Unwashed, resting cultures of these crude isolates were assayed for their rate of sulfate reduction (Fig. 1). The isolates with the highest rates of sulfate reduction, such as C, D, and E, were plated out on roll tubes and resolated repeatedly. They were all gram-negative straight rods that were morphologically similar. Repeated isolations were necessary to separate strain D from a large gram-positive rod, which grew only when a glucose energy source was used and did not appear when lactate was the

![Fig. 1. Rate of sulfate reduction to sulfide of five isolates from the rumen.](image-url)
substrate. This gram-positive rod, after isolation from strain D, did not reduce appreciable amounts of sulfate and has been identified as *Bacillus cereus* (C. Kepler and S. Tove, personal communication).

The gram-negative, sulfate-reducing isolate, strain D, was checked for cultural purity when grown on either lactate or glucose as the sole carbon source, using both solid and liquid media. This isolate, carried in either lactate or glucose media and then each plated out on both glucose and lactate solid media, gave rise to black colonies only. These colonies, when observed microscopically with the Gram stain, contained only the gram-negative rod described here. Upon repeated subculture in sulfate media containing glucose or other carbohydrates, the organism looked thinner and one end of the cell stained more deeply when observed by Gram stain, giving the cells a somewhat club-shaped appearance. A 10⁴ dilution of the organism previously grown in lactate was plated in petri dishes onto both lactate and glucose agar, in triplicate, and incubated under H₂ and CO₂ in the BBL Anaerobic GasPak jar. Similar counts were obtained on both lactate (9 ± 2 colonies per plate) and glucose (10 ± 1 colonies per plate). The colonies on glucose agar were somewhat smaller than those on lactate; however, the organisms were morphologically similar as seen by the Gram stain. The almost identical counts on either lactate or glucose agar plates is strong evidence that this is a pure culture since the inoculum was taken from an overnight culture which had been grown in lactate medium alone for several weeks, being transferred daily. Therefore, it is improbable that any glucose-utilizing contaminant could be present in the same numbers as the lactate-utilizing sulfate reducer. Rather, the data show that this is a pure culture capable of utilizing either lactate or glucose.

**Characterization.** The bacterium is an obligately anaerobic, gram-negative, straight rod, about 3 to 6 μm by 1 μm (Fig. 2 and 3). The organism is not motile under the conditions described here, and no evidence of flagella was found although several stains were used (4, 9, 23). Spore stains were made on cultures at different stages of growth, as well as after heat and oxygen treatments, and all results were negative. The bacterium grows well at 37 C, but no growth occurs at 25 or 45 C.

To determine the growth of this organism with certain taxonomically significant carbon sources, the procedures described by Postgate and Campbell (30) were followed with the exceptions in preparation of the media as described above. Growth was obtained in the presence of sulfate, with lactate, pyruvate, glucose, fructose, galactose, ribose, and mannose as sole carbon sources; formate, acetate, propionate, butyrate, malate, and choline gave no growth. In the absence of sulfate, glucose and pyruvate were utilized as sole carbon sources, whereas lactate and choline gave no growth. Upon microscopic examination of Gram stains of those cultures where growth was obtained, the cells appeared morphologically identical as described above.

The buoyant density of DNA isolated from this organism was 1.725 g/cm³, giving a guanosine plus cytosine (G+C) percentage of 61.2 when calculated by Sueoka's method (34). These values are similar to the G+C percentages reported by Saunders et al. (33) with *D. vulgaris*, *D. agricanus*, and *D. gigas*. H. E. Jones (16) has more recently described the isolation of a nonsporulating *Desulfovibrio* that has a similar G+C percentage.

Whole cells, upon the addition of 2 N NaOH, fluoresced pink under ultraviolet light, indicating the presence of the pigment desulfoviridin (28). Cell-free extracts showed the characteristic absorption of cytochrome c₅ at 525 and 553 nm when reduced and of desulfoviridin at 630 nm, under both oxidizing and reducing conditions.

Washed cell suspensions and cell-free extracts reduced methyl viologen in the presence of hydrogen, but not in its absence, indicating the presence of hydrogenase. This did not occur with boiled controls.

Nitrate was not reduced in the presence of sulfate when determined under the conditions described by Jones (16).

The initial rate of sulfate reduction to sulfide observed with growing cells of this bacterium was 38 μmol of S²⁻ formed per h per mg of protein. This rate is considerably higher than that previously reported for *D. desulfuricans* (15).

**Activity of enzymes in sulfate reduction.**

Cell-free extracts of our isolate were assayed for two enzymes involved in sulfate reduction. The first enzyme, ATP sulfurylase, which activates sulfate by ATP to form APS, is widespread in nature but occurs at a much higher specific activity in bacteria which produce massive amounts of sulfide from sulfate (dissimilatory sulfate reduction). This enzyme will also catalyze the rapid liberation of inorganic phosphate from ATP in the presence of inorganic pyrophosphatase and group VI anions such as mo-
Fig. 2. Light micrograph of a Gram stain of the isolate. Bar indicates 5 μm.
Fig. 3. Electron micrograph of the isolate negatively stained with phosphotungstic acid. Bar indicates 1 μm.
lybdate, through the proposed formation of an unstable AMP-anion anhydride (37). The net ATP and MoO$_4^{2-}$-dependent ATP sulfurylase activity of this isolate was 75 (± 1) μmol of inorganic phosphate per h per mg of protein. This activity is considerably higher than that reported for yeast (37) and Penicillium chrysogenum (35), two organisms which reduce sufficient sulfate for incorporation into cellular material (assimilatory sulfate reduction). This specific activity is comparable to the ATP-sulfurylase activities reported by Akagi and Campbell (1) for the dissimilatory anaerobic sulfate-reducing bacteria, D. desulfuricans and Clostridium nigrificans (Desulfotomaculum nigrificans). Essentially, no inorganic phosphate was liberated in this assay in the absence of cell-free extract.

The second enzyme assay, APS-reductase, has been found only in the dissimilatory sulfate-reducing bacteria, Desulfovibrio and Desulfotomaculum, and in some thiobacilli which oxidize sulfide to sulfate (27). This enzyme reduces APS to sulfite and AMP. Cell-free extracts of our isolate had a specific activity of APS-reductase of 5.7 (± 0.2) μmol of APS reduced per h per mg of protein. Peck et al. (27) reported a specific activity of 4.8 μmol of APS reduced per h per mg of protein for crude cell-free extracts of D. desulfuricans, using the same method of assay. The reduction of ferricyanide in this assay was found to be dependent on each of the following: cell-free extract, AMP, and sulfite.

**Identification of a sulfur-containing nucleotide.** When cell-free extracts of our bacterium were incubated with ATP and ³⁵S-labeled sulfate, a radioactive nucleotide was formed that had the same electrophoretic mobility as APS (Fig. 4). Upon hydrolysis of this nucleotide by 0.1 M HCl at 37°C for 30 min and chromatography, only AMP was detected. APS is the only sulfur-containing nucleotide known to hydrolyze to AMP under these conditions (31). The formation of this nucleotide is dependent on the presence of each of the following in the reaction mixture: cell-free extract, ATP, and SO$_4^{2-}$. The observed inhibition by MoO$_4^{2-}$, and simultaneous qualitative increase in the amount of AMP formed, is further evidence that the radioactive nucleotide formed is APS, the product of the molybdate-inhibited ATP-sulfurylase. Under the electrophoretic conditions used in this experiment, 3'-phosphoadenosine-5'-phosphosulfate (PAPS) has a mobility slightly greater than ATP. A nucleotide with this mobility was not observed on

![Electrophoresis of nucleotides.](image-url) **DISCUSSION**

The rumen fluid used in this study contained approximately 10$^7$ sulfate-reducing organisms per ml compared to the previously reported counts of only 10$^6$ sulfate reducers per ml (8). The high percentage of sulfate-reducing bacteria present in our animals facilitated isolation so that it was unnecessary to preheat the rumen fluid and thereby select for spore formers. Microscopic inspection of our isolates with high rates of sulfate reduction revealed that most were gram-negative, straight rods similar to the isolate, strain D, described here. These facts suggest that the organism we have isolated makes a major contribution to the total sulfate reduced in the rumen of sheep fed sulfate-containing diets.

Although sulfate reduction is known to occur at low levels in a wide variety of organisms in order to provide reduced sulfur compounds for incorporation into cellular material (assimilatory sulfate reduction), only bacteria of the genera Desulfovibrio and Desulfotomaculum are known to utilize sulfate as a terminal electron acceptor in the production of large amounts of hydrogen sulfide which is released from the cell (dissimilatory sulfate reduction). Peck (26), in a review of these two types of sulfate reduction, points out that one major difference between them is that the assimilatory pathway requires 2 mol of ATP to activate 1 mol of sulfate to PAPS before reduction of the sulfate, whereas the dissimilatory pathway requires only one ATP to activate sulfate to APS,
which is then reduced by APS-reductase without further activation.

Cell-free extracts of this isolate contained ATP-sulfurylase as well as APS reductase at specific activities comparable to those of other dissimilatory sulfate-reducing bacteria examined to date. APS and not PAPS was identified as an intermediate in the reduction of sulfate in this organism when cell-free extracts were incubated with ATP and radioactive sulfate. APS was not formed, however, when molybdate was included in the reaction mixture. These observations together with the overall high rate of sulfate reduction observed would indicate that the pathway of sulfate reduction in this bacterium is similar to that of other dissimilatory sulfate-reducing bacteria whose pathways have been studied.

A comparison of the characteristics of our isolate with those previously described for species of the two genera of sulfate-reducing bacteria, Desulfovibrio and Desulfotomaculum, shows that it is more similar to the genus Desulfovibrio, yet it differs in several important respects from any species previously described.

The properties of our isolate correspond to those listed by Campbell and Postgate (30) as taxonomically significant for classification in the genus Desulfovibrio in that it is nonsporulating, contains both desulfoviridin and cytochrome c₅₅, and has a DNA base composition of 61.2% G+C. Desulfovibrio species generally show some type of curvature, either vibroid, sigmoid, or spiriloid; however, Jones (16) has reported the isolation of a nonsporulating sulfate-reducing bacterium which is a straight rod. The isolate described here always appeared as a straight rod in all of the media in which it grew and only varied in its width or length. This bacterium also differed from species of both Desulfovibrio and Desulfotomaculum with respect to motility. We were unable to observe motility or flagella, although a wide variety of tests were used. Postgate and Campbell (30) do note that two nonmotile strains of Desulfovibrio vulgaris have been reported.

This is, to our knowledge, the first Desulfovibrio sp. isolated from the rumen, although Lewis (18) proposed in 1954 that such organisms should be present in the rumen and attempted to isolate them without success. A formal description of this organism (isolate strain D) follows.

Straight rods, 3 to 6 μm by 1 μm, nonmotile, nonsporulating, and gram negative.

Deep agar colonies, black, particularly if iron salts are present in the media.

Obligate anaerobe, mesophilic. Utilizes lactate, pyruvate, glucose, fructose, galactose, ribose, and mannose but not formate, acetate, propionate, butyrate, malate, or choline as carbon sources in the presence of sulfate; in the absence of sulfate, growth occurs with glucose and pyruvate, but not with lactate and choline.

Whole cells reduce sulfate to sulfide.

Whole cells fluoresce pink under ultraviolet light immediately after the addition of a few drops of 2.0 N NaOH, indicating the presence of desulfoviridin.

Cell-free extracts show characteristic absorption bands of cytochrome c₅₅ at 525 and 553 nm and of desulfoviridin at 630 nm.

Contains hydrogenase, ATP-sulfurylase, and APS-reductase.

DNA base composition is 61.2% G+C. Isolated from the rumen fluid of a sheep fed a purified diet containing urea as the nitrogen source and sodium sulfate as the sole dietary source of sulfur.

Subcultures deposited with the American Type Culture Collection, Rockville, Md., as ATCC 27882.

ACKNOWLEDGMENTS

The authors appreciate the outstanding technical assistant R. McKinley provided in the isolation of this organism.

Supported in part by grants from Public Health Service Research grant no. AM 13055 from the National Institute of Arthritis, Metabolism, and Digestive Diseases, and the Herman Frasch Foundation.

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