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Taurine Reduction in Anaerobic Respiration of *Bilophila wadsworthia* RZATAU

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Organosulfonates are important natural and man-made compounds, but until recently (T. J. Lie, T. Pitta, E. R. Leadbetter, W. Godchaux III, and J. R. Leadbetter. Arch. Microbiol. 166:204–210, 1996), they were not believed to be dissimilated under anoxic conditions. We also chose to test whether alkane- and arenesulfonates could serve as electron sinks in respiratory metabolism. We generated 60 anoxic enrichment cultures in mineral salts medium which included several potential electron donors and a single organic sulfonate as an electron sink, and we used material from anaerobic digestors in communal sewage works as inocula. None of the four aromatic sulfonates, the three unsubstituted alkanesulfonates, or the *N*-sulfonate tested gave positive enrichment cultures requiring both the electron donor and electron sink for growth. Nine cultures utilizing the natural products taurine, cysteate, or isethionate were considered positive for growth, and all formed sulfide. Two clearly different pure cultures were examined. Putative *Desulfovibrio* sp. strain RZACYSA, with lactate as the electron donor, utilized sulfate, aminomethanesulfonate, taurine, isethionate, and cysteate, converting the latter to ammonia, acetate, and sulfide. Strain RZATAU was identified by 16S rDNA analysis as *Bilophila wadsworthia*. In the presence of, e.g., formate as the electron donor, it utilized, e.g., cysteate and isethionate and converted taurine quantitatively to cell material and products identified as ammonia, acetate, and sulfide. Sulfite and thiosulfate, but not sulfate, were utilized as electron sinks, as was nitrate, when lactate was provided as the electron donor and carbon source. A growth requirement for 1,4-naphthoquinone indicates a menaquinone electron carrier, and the presence of cytochrome *c* supports the presence of an electron transport chain. Pyruvate-dependent disappearance of taurine from cell extracts, as well as formation of alanine and release of ammonia and acetate, was detected. We suspected that sulfite is an intermediate, and we detected desulfoviridin (sulfite reductase). We thus believe that sulfonate reduction is one aspect of a respiratory system transferring electrons from, e.g., formate to sulfite reductase via an electron transport system which presumably generates a proton gradient across the cell membrane.

Fuchs et al. (16) found no evidence in the literature for a desulfonation reaction in anaerobic bacteria, although biotransformations distant from the sulfonate group are well established (7, 28). Thus, despite a desulfonation reaction (of bromoethanesulfonate) by methyl coenzyme M reductase (21), desulfonation was firmly associated with aerobes (15, 25, 40) and usually involved oxygenation directly (14, 23, 32, 43, 49).

The first data indicating the occurrence of desulfonation under anoxic conditions involved assimilation of sulfonate sulfur (6) and were confirmed by Denger et al. (11), who showed quantitative assimilation of sulfur from a range of arenesulfonates by the strict anaerobe *Clostridium beijerinckii* EV4. We proceeded to confirm the ease with which anaerobes can be isolated to assimilate sulfur not only from inert arenesulfonates but also from inert alkanesulfonates (10). In no case, however, was desulfonation detected in cell extracts, and so we turned to dissimilatory reactions, with their higher specific activities at a given growth rate, in the hope of elucidating a mechanism of anoxic desulfonation.

Taurine (Fig. 1) is the major organic solute in humans; it is derived from cysteine (as is cysteate) and can be transformed to isethionate (22). The concept of organosulfonates as electron acceptors has been established in a review (39), and the first experimental data have been presented (31). Independent of that work, we prepared enrichment cultures to utilize sulfonates as electron acceptors for growth. We now report the isolation of a taurine-reducing organism, identified as *Bilophila wadsworthia*, which, in the presence of an external source of electrons, converts taurine to ammonia, acetate, and sulfide. Reactions in cell extracts of *B. wadsworthia* bear some analogies to the degradation of taurine in aerobes (25, 40).

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MATERIALS AND METHODS

Materials. The sulfonates studied were commercial products of >99% purity and were used as supplied. The sources, except for cysteate and isethionate (Fluka, Buchs, Switzerland), have been given elsewhere (10). Other chemicals were of reagent grade or better and were obtained from Fluka or from Merck-Schuchardt (Munich, Germany). Gases were of high purity (>99.999%; Sauerstoffwerk, Friedrichshafen, Germany). The inocula for enrichment cultures were from the anaerobic digestors of two communal wastewater treatment plants (Konstanz and Radolfzell in Germany).

Growth media and enrichment cultures. The freshwater mineral salts medium of Widdel and Pfennig (48) was used but routinely with NH_4Cl at 0.5 mM (or 4.7 mM with isethionate). This medium was buffered with 50 mM $NaHCO_3$ and supplemented with a selenite-tungsten solution (44), a trace element solution SL 10 (47), a seven-vitamin solution (34), 2 μM resazurin, and, as the reductant, Ti(III) nitrilotriacetate (routinely to about 1 mM) (11). The routine medium contained 0.2 mM sulfide as the source of sulfur, 200 μg of 1,4-naphthoquinone per liter, formate (e.g., 40 mM) as the source of electrons, and taurine (e.g., 10 mM) as the electron sink, and the inoculum was 10%. The pH was brought to 7.0 with HCl or Na_2CO_3 prior to inoculation. All cultures were incubated at 30°C under an atmosphere of N_2 plus CO_2 (80:20) in serum bottles sealed with butyl rubber septa.

Several variants of the medium were used in the enrichment cultures. NH_4Cl was used at 4.7 mM, and the sulfur supply was 0.1 mM sulfate plus 0.2 mM sulfide. The naphthoquinone was not present. Some enrichments were done with 0.5 mM reductant, but no advantage was detected. Three different sets of electron sources were used: (i) 10 mM formate, 10 mM ethanol, and 10 mM DL-lactate; (ii) 10 mM acetate, 10 mM propionate, and 10 mM butyrate; and (iii) 10 mM acetate, 10 mM ethanol, and 10 mM DL-lactate. Mostly, the sulfonate was

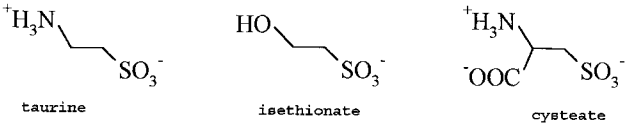

FIG. 1. Structures of taurine, cysteate, and isethionate.

present at 20 mM (except for 10 mM 2,6-naphthalenedisulfonate); no advantage was found in halving these concentrations. The inoculum (5%, vol/vol) was taken from the anaerobic digestor. The first positive enrichments were visible within about a week, whereas others required several weeks. On serial subculturing into homologous medium, the fastest cultures were outgrown in 2 days.

Organisms were isolated by the agar shake method (35) with selective medium. In one case, we obtained a pure culture without altering the medium. In two other cases, many possible supplements had to be tested: outgrown medium from an enrichment culture grown without the external sources of electrons (passed through a sterile 0.2-μm-pore-size membrane filter), sterilely filtered fermented pig manure (46), 0.05% yeast extract, 0.05% Casamino Acids, 50 μg of α-lipoic acid per liter, 200 μg of 1,4-naphthoquinone per liter, 50 μg of hemin per liter, and 20 μM putrescine. Apart from the enrichment medium, only the naphthoquinone had a significant effect. The purity of the isolated bacteria was tested microscopically and in selective shake cultures. The shake cultures were then supplemented with 0.1% yeast extract, and the homogeneity of the colonies was examined. Liquid cultures were examined with AC medium (at 10% of the concentration suggested by the manufacturer [Difco, Detroit, Mich.]) supplemented with the electron donor and electron acceptor to detect possible contaminants. Possible aerobic growth of organisms was tested on oxic blood agar plates (Becton Dickinson, Heidelberg, Germany).

Stock cultures were maintained at 4°C. Strain RZATAU has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) as *B. wadsworthia* RZATAU with the accession no. DSM 11045.

Growth experiments and work in cell-free systems. Pure cultures were routinely grown in 25-ml portions of medium in 36-ml serum bottles under an atmosphere of N_2 plus CO_2 (80:20) and closed with a butyl rubber stopper. Substrate ranges and mass balances were examined in replicates in 17-ml Hungate tubes containing 10 ml of medium with the standard gas phase and stopper; the inoculum was 10% (vol/vol). Growth experiments were done under the same conditions but with 100 ml of medium in 150-ml infusion bottles, and samples were removed through the stopper with a syringe at intervals; the inoculum was 1%. Cells from 100-ml cultures were harvested (2,100 × *g* for 20 min at 4°C) anoxically in the culture vessel at the end of exponential growth. The supernatant fluid was removed through the stopper with a syringe under a stream of N_2, and the cells were washed twice in 100 mM Tris-HCl buffer (pH 8.0). The pellet was suspended in 2 ml of the Tris-HCl buffer and transferred to an anoxic, 8-ml serum bottle on ice. If cell extracts were required, a chilled 3.7-ml French pressure cell fitted with a needle on the inlet/outlet was flushed with N_2 and the needle was inserted into the 8-ml serum bottle. After transfer of the suspension into the pressure cell, bacteria were disrupted by three anoxic passages (140 MPa) through the cell. Intact bacteria and debris were removed by centrifugation in the 8-ml serum bottle (2,000 × *g* for 10 min at 4°C).

Experiments with cell suspensions were done at 30°C (in a water bath) with 3-ml reaction mixtures in anoxic 6-ml tubes under the standard gas phase and closed with butyl rubber septa. The reaction mixture contained 200 μmol of Tris-HCl buffer (pH 8.0), 15 μmol of taurine, 60 μmol of formate, and cells (about 0.5 mg of protein), with which the reaction was started. Controls were done without cells, with boiled cells, or without formate. Samples were taken at intervals with a gas-tight syringe and frozen immediately.

Experiments with cell extracts were done under conditions similar to those with cell suspensions, except that a 2-ml reaction mixture was used. Initial experiments, directly analogous to the work with whole cells, gave no reaction, so that a reductant [Ti(III) nitrilotriacetate; 2 μmol] and an alternative buffer (100 mM potassium phosphate [pH 7.0]) were tried, without success. We then tried combinations of formate, pyruvate, 2-oxoglutarate, NADH, coenzyme A, flavin adenine dinucleotide, ATP, pyridoxal phosphate, thiamine pyrophosphate, vitamin B_{12}, and components of the growth medium. The standard reaction mixture became 200 μmol Tris-HCl buffer (pH 8.0), 10 μmol of taurine, 10 μmol of pyruvate, 1 μmol of NAD^+, and up to 0.5 mg of protein, with which the reaction was started. Controls were done without cell extract, with boiled extract, or without taurine, pyruvate, or NAD^+. Samples were taken at intervals with a gas-tight syringe and frozen in liquid N_2 immediately.

Analytical methods. Reversed-phase chromatography was done as described elsewhere (30). Taurine was quantified by high-pressure liquid chromatography after derivatization with 2,4-dinitrofluorobenzene (12). We occasionally confirmed these data by derivatizing with *o*-phthaldialdehyde (essentially as in reference 41). Alanine was detected after derivatization with 2,4-dinitrofluorobenzene (see above) and by reaction with alanine dehydrogenase (4). Acetate was determined and tentatively identified by gas chromatography on a 2-m by 2-mm glass column packed with 60/80 mesh Carbopack C coated with 0.3% Carbowax 20M–0.1% H_3PO_4, as specified by the manufacturer (Supelco). The identity of acetate was confirmed by the specific acetyl coenzyme A synthase assay (4). The ammonium ion concentration was measured by a colorimetric assay (Berthelot) (18) and tentatively identified by flow injection analysis (20), and the identification was confirmed by reaction with glutamate dehydrogenase (4). Sulfide was determined by the formation of methylene blue (8); the formation of a black precipitate with iron salts and, more commonly, of a brown precipitate with copper salts (46) was used to confirm the identity of the ion. Formate was determined colorimetrically (29). Sulfite, stabilized with formaldehyde, was measured by ion chromatography with the column and apparatus indicated previously (30); a colorimetric method (27) was used occasionally. Sulfite reductase (desulfoviridin) was detected in extracts of oxically disrupted cells as an absorbance band at 630 nm and by its red fluorescence when irradiated with UV light at 366 nm (36, 37). 16S rDNA was sequenced by the Deutsche Sammlung von Mikroorganismen und Zellkulturen, and the data were used to generate a dendrogram (12). The protein in whole cells was assayed by a Lowry-type method (9), whereas protein in cell extracts was assayed by the method of Bradford (5); the standard was bovine serum albumin in each case. The Gram reaction was assayed by the KOH test (19). Catalase and oxidase were measured by routine tests (17). Cytochromes were assayed photometrically in cell extracts as difference spectra between the air-oxidized and dithionite-reduced forms. Turbidity was assayed in a photometer set at 580 nm.

Nucleotide sequence accession number. The sequence data are available in the NCBI GenBank library under accession no. U82813.

RESULTS

A total of 60 strictly anaerobic enrichment cultures (resazurin indicator colorless) were set up in media containing mixtures of electron donors and a single organosulfonate as a potential electron sink. The 30 pairs (the members of each pair were identical except for the inoculum) were in three groups (different electron sources) of 10. Only the substituted alkanesulfonates taurine (six of six enrichments), cysteate (six of six), and isethionate (two of two) and the *N*-sulfonate cyclamate(*N*-cyclohexylsulfanilic acid) (one of six) gave positive enrichments, which were initially classified as growth equivalent to a positive control (sulfate or sulfite as the electron sink) together with no growth in a negative control (no electron sink added); this growth was usually accompanied by a black precipitate attributed to iron sulfide. Methane-, ethane-, and heptanesulfonates (each zero of six), benzene- and toluenesulfonates (each zero of six), sulfobenzoate (zero of four) and naphthalenedisulfonate (zero of six) did not enable visible growth.

In general, cultures with the fermentable electron sources were quicker to give positive cultures (1 week) than were those with the nonfermentable sources (up to 4 weeks). After three to five transfers, we examined 13 representative cultures to determine whether both the putative electron sink and electron donor were required for significant growth. One putative cysteate enrichment and the cyclamate enrichment were eliminated; the putative electron source alone supported growth, negligible sulfide was released, and, where measured, negligible sulfonate disappeared in the presence of the putative electron source. One taurine enrichment and one cysteate enrichment were eliminated as fermenting the sulfonate; they will be described elsewhere. Therefore, nine cultures that tentatively were able to reduce three sulfonates (taurine, cysteate, or isethionate) were obtained. Each released sulfide from the sulfonate and, where tested, caused disappearance of the sulfonate.

The three fastest-growing cultures, two utilizing taurine and one utilizing cysteate, were then used to obtain pure isolates. The organism RZACYSA was isolated most easily, because no supplementation to the standard growth medium was required for growth. Strain RZACYSA was tentatively identified as a *Desulfovibrio* sp. (Fig. 2). The organism utilized sulfate, cysteate, taurine, isethionate, or aminomethanesulfonate as an electron sink with DL-lactate as an electron donor, and it released the tentatively identified acetate, ammonia, and sulfide from

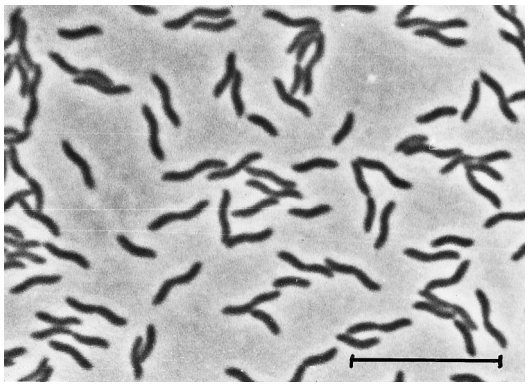

FIG. 2. Phase-contrast photomicrograph of strain RZACYSA, utilizing 10 mM cysteate as an electron acceptor with 20 mM lactate as the electron donor. The strictly anaerobic, motile, gram-negative organism was tentatively identified as a *Desulfovibrio* sp. Bar, 10 μm. When aminomethanesulfonate was used by strain RZACYSA as the electron aceptor, extremely long spirilla (up to 500 μm) were formed during the early phase of growth, which was very slow.

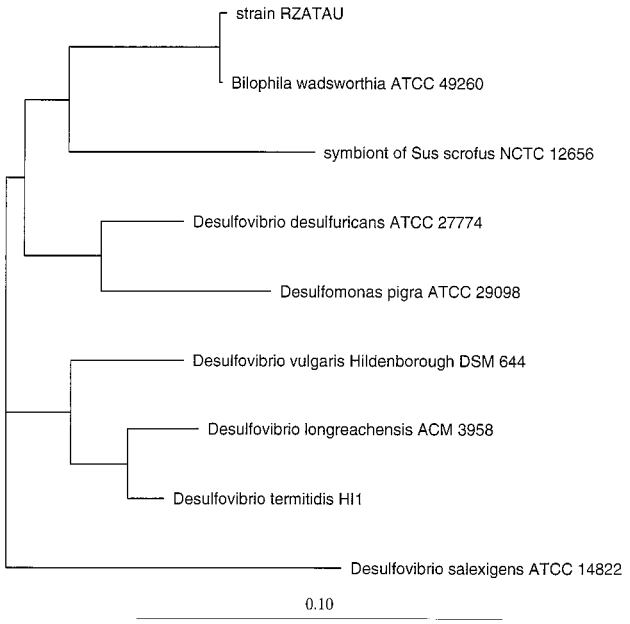

FIG. 4. Dendrogram of the phylogeny of strain RZATAU near the *Desulfovibrio* group based on 16S rDNA sequences. The similarity value to *B. wadsworthia* ATCC 49260^T is 99.8% (sequence L35148). The next closest similarity value is 91.5% to *D. desulfuricans* ATCC 27774. The scaling indicates 10 nucleotide substitutions per 100 nucleotides.

cysteate. We did not examine this isolate further, because of similarities to work in progress elsewhere (31).

Culture RZATAU, at appropriate dilutions in agar shakes, displayed some central yellow colonies surrounded by many satellite colonies which rapidly blackened. We presumed that the latter colonies were the organisms of direct interest, because their morphology corresponded to the predominant form seen in enrichment cultures; short, nonmotile rods, which were found to be gram negative, oxidase negative, and catalase positive (Fig. 3). Strain RZATAU and the essentially identical strain KNATAU grew very poorly, if at all, in selective medium. However, when filter-sterilized, outgrown medium from modified enrichment cultures (no external electron donor added) was added to the selective medium, significant growth of the isolate was obtained. The supplement requirement to basal medium was then shown to be solely 1,4-naphthoquinone. Only strain RZATAU has been studied in detail.

Strain RZATAU was identified after sequencing about 95% of its 16S rDNA. Analysis of the sequence showed 99.8% identity to *B. wadsworthia*, which clusters with the sulfate-reducing organisms (Fig. 4). The identification was supported by all the physiological observations made.

B. wadsworthia RZATAU was found to utilize at least seven oxosulfur compounds as electron sinks: sulfite, thiosulfate, dimethyl sulfoxide, dimethyl sulfone, taurine, cysteate, and isethionate. Many other compounds, including sulfate, sulfur, the other sulfonates used in enrichment cultures, coenzyme M, aminomethanesulfonate, and 3-cyclohexylaminopropanesulfonate (CAPS) and *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) buffers, were not utilized as electron acceptors. Nitrate but not fumarate served as an electron acceptor with lactate as the electron donor and carbon source. We examined taurine in the greatest detail and found that many electron donors could be utilized by the organism: formate, hydrogen, DL-lactate, pyruvate, succinate, fumarate, DL-malate, ethanol, glycerol, *n*-propanol, and *n*-butanol. Of the carbon-containing compounds, only pyruvate could serve as a substrate for fermentative growth. The following compounds did not serve as electron donors: acetate, propionate, butyrate, benzoate, glucose, fructose, xylose, arabinose, crotonate, and methanol. The organism could utilize acetate as the source of carbon.

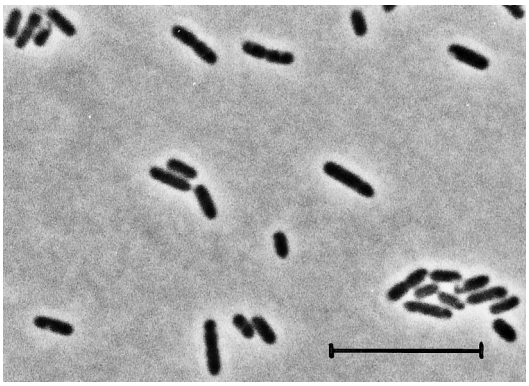

FIG. 3. Phase-contrast photomicrograph of *B. wadsworthia* RZATAU, utilizing 10 mM taurine with 40 mM formate as the electron donor. Bar, 10 μm.

Growth of strain RZATAU with taurine, cysteate, or isethionate yielded ammonia (where appropriate), acetate, and sulfide, and this was examined in detail with taurine. Ammonium ion was tentatively identified in the Berthelot colorimetric test, and the identification was supported by flow injection analysis and established by the specific reaction with glutamate dehydrogenase. Acetate was tentatively identified by cochromatography in a gas chromatograph, and the identification was established enzymatically. Sulfide was tentatively identified as a black precipitate with iron in the medium; the identification was supported by a spot test (a brown precipitate with a copper salt) and confirmed by the generation of methylene blue (8).

Strain RZATAU grew exponentially ($\mu = 0.15\ h^{-1}$) with taurine as the electron sink (Fig. 5A) and formate as the electron donor, and these substrates were utilized concomitantly with growth. The specific degradation rate of taurine was calculated as 2.7 mkat/kg of protein. The products, ammonia, acetate, and sulfide, were formed concomitantly with growth

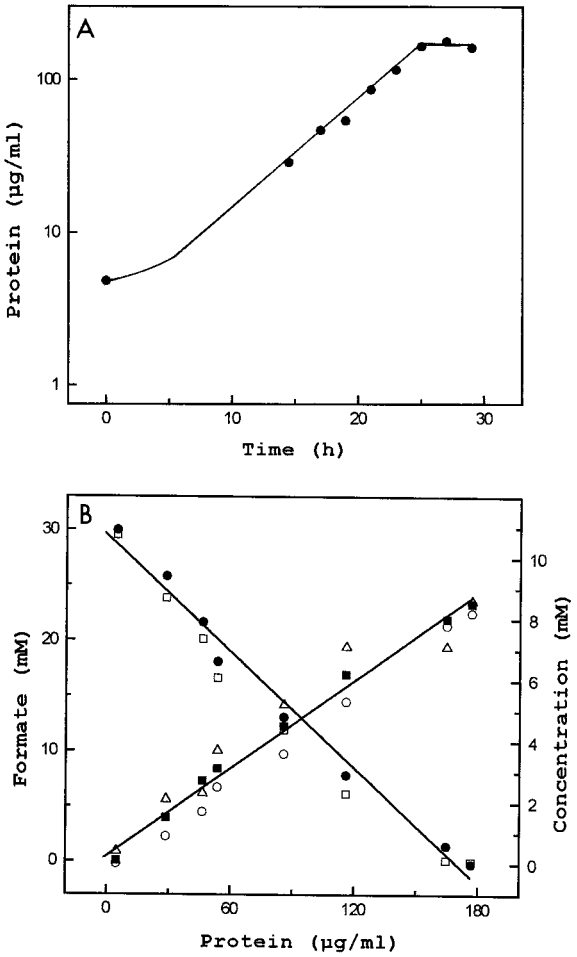

FIG. 5. Growth of *B. wadsworthia* RZATAU in naphthoquinione-supplemented standard medium containing 10 mM taurine and 30 mM formate. The growth curve (A) is depicted in linearized form (B) to indicate the stoichiometry of substrate utilization and product formation. Symbols in panel B: ●, taurine; □, formate; ○, acetate; ■, sulfide; △, ammonia.

(Fig. 5B). The molar growth yield under these conditions was 15 g of protein/mol of taurine. In a complementary experiment under different conditions and allowing for assimilation during growth, the taurine nitrogen and sulfur balances were 89 and 83%, respectively, and the electron balance was 96% (Table 1). Suspensions of nongrowing cells degraded taurine at a specific rate of 1.0 mkat/kg of protein; the mass balances for carbon, nitrogen, and sulfur were 100, 96, and 75%, respectively. We

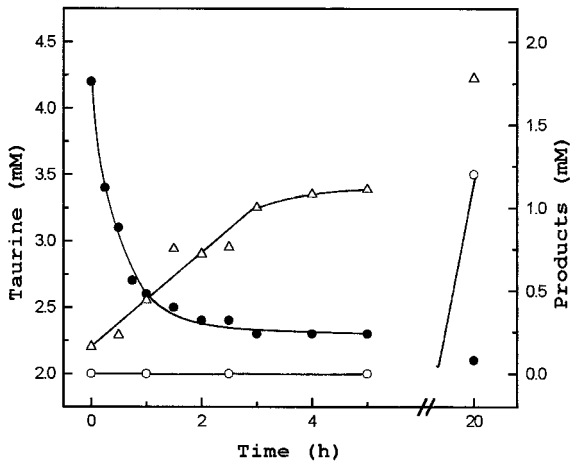

FIG. 6. Transformation of taurine to products in cell extracts of *B. wadsworthia* RZATAU. Symbols: ●, taurine; ○, acetate; △, ammonia.

presume that some of the volatile sulfide was lost on sampling. There is thus no significant unknown product.

Some of the enzymatic reactions involved in the reduction of taurine were observed in crude extracts. The disappearance of taurine from reaction mixtures depended on the addition of pyruvate, when a specific rate of 1.7 mkat/kg of protein was observed, although the reaction was incomplete (Fig. 6); there was no disappearance in the absence of pyruvate. The formation of alanine was detected (data not shown), as was the release of ammonium ions, which was accelerated in the presence of NAD^+. Ammonia was released at about 0.4 mkat/kg of protein, and acetate was formed very slowly (Fig. 6). No sulfide was detected. Sulfite was also not detected, although it could be measured if added to the mixture. Sulfite reductase, detected as desulfoviridin, was found in cell extracts. We could detect no effect of formate, as the electron donor, on the disappearance of taurine or the formation of sulfide or sulfite; in colorimetric tests, there was no alteration in the absorption of reduced benzyl viologen or methyl viologen supplied as a possible electron donor.

Redox difference spectra of crude extracts of strain RZA TAU gave absorption bands at 549, 520, and 414 nm, which indicated a *c*-type cytochrome (standard values, 550, 521 and 415 nm [45]). Cytochrome *c* was present at about 3 mg/g of protein, assuming a molecular mass of 12 kDa and ε_{550} of 21,000 mol^{-1} cm^{-1} (13).

DISCUSSION

Our basic aim was to find dissimilatory anaerobic processes involving organosulfonates. Our use of a medium with a pow-

TABLE 1. Representative mass balances during growth of *B. wadsworthia* RZATAU with limiting taurine and excess formate[a]

Mean amt (µmol) of substrate utilized ± SEM		Mean amt of product released ± SEM				Amt (µmol) of products assimilated			Recovery (%)			Molar growth yield (g of protein/mol of taurine)
Taurine	Formate	Protein (mg)	Acetate (µmol)	Ammonia (µmol)	Sulfide (µmol)	Formate	Acetate	Ammonia	Electrons	Nitrogen[b]	Sulfur[b]	
54	134 ± 12	0.66	38 ± 6	35 ± 3	45 ± 0.5	25.6	20.9	13.2	96	89	83	12

[a] We considered that 15.8 µmol of acetate and 19.4 µmol of formate were required to form 1 mg of cell dry matter (see the second equation in the Discussion). Protein was considered to represent 50% of cell dry matter. The nitrogen content of the cell was considered to be 14% (33). Assimilation of sulfur from taurine was considered to be negligible.

[b] From taurine.

erful reducing agent in the presence of a redox indicator, which showed that we maintained reducing conditions, and the inability of the organism(s) to grow aerobically shows that we obtained strictly anaerobic bacteria. Correspondingly, the isolates were identified as *Bilophila* (Fig. 3 and 4) or tentatively attributed to *Desulfovibrio* (Fig. 2).

Our evidence for the dissimilation of, e.g., taurine is fourfold. The substrate disappears quantitatively during growth and concomitantly with growth (Fig. 5B). Three products, ammonia, acetate, and sulfide, are formed concomitantly with growth and substrate utilization (Fig. 5B). They have been conclusively identified. The yields of these products, especially if examined in cell suspensions (Table 1), are usually about 100% (except for the volatile sulfide), which indicates that no significant product was overlooked. In addition, there is a complete electron balance (Table 1).

We believe the nature of the dissimilation to be respiratory. The sulfonate is not utilized by *B. wadsworthia* RZATAU in the absence of an external source of electrons, and with the exception of pyruvate, none of these sources of electrons can be fermented by the organism. Growth is dependent on a supplement, 1,4-naphthoquinone, a precursor of menaquinone, which suggests the presence of an electron transport chain. Direct evidence for a respiratory chain is provided by the presence of cytochrome *c*.

We considered growth to occur, formally, as two basic processes: dissimilation

$$C_2H_7NSO_3 + 2HCO_2^- + H^+ \rightarrow C_2H_3O_2^- + 2CO_2 + HS^- + NH_4^+ + H_2O$$

(where $\Delta G^{\circ\prime} = -211.3$ kJ/mol of taurine [values from reference 42]) and assimilation

$$13C_2H_3O_2^- + 16HCO_2^- + 25H^+ \rightarrow 8<C_4H_7O_3> + 10CO_2 + 10H_2O + 4OH^-$$

where $<C_4H_7O_3>$ is considered to be a monomer of biomass. The $\Delta G^{\circ\prime}$ value indicates a maximum energy yield of about 3 mol of ATP/mol of taurine, given a requirement of 60 to 80 kJ/mol of ATP (42). The growth molar yield, about 24 to 30 g (dry weight)/mol of taurine (Fig. 4; Table 1), thus implies a practical ATP yield (Y_{ATP}) of about 8 to 10 g (dry weight)/mol, which is close to the Y_{ATP}^{max} values of 11.4 to 14.6 g (dry weight)/mol (1). There would thus appear to be very effective energy coupling in this anaerobic respiration, but we only derived the dry weights used in the calculation (Table 1), so the value is probably not unusual.

The anaerobic dissimilation of an organosulfonate is novel (39). The first anoxic desulfonations in the assimilation of sulfur have only just been established (6, 10, 11). The idea of sulfonate respiration has been discussed (39), and the corresponding research shows reduction of isethionate (31) to acetate and sulfide in several sulfate reducers, especially *Desulfovibrio desulfuricans* IC1. We suspect that we also isolated a *Desulfovibrio* sp. (strain RZACYSA [Fig. 2]), although with a broader substrate range than strain IC1. We concentrated, however, on a complete set of data to establish unequivocally the reduction of taurine by *B. wadsworthia* RZATAU (Fig. 5; Table 1). We suspect the reaction to be widespread in this species, because taurine and taurine conjugates enhance the growth of isolates of the organism, from clinical samples, on complex medium and cause the release of sulfide (38), and clinical isolates are indistinguishable from strain RZATAU in our medium (30a). Given that taurine is the most abundant organic solute in mammals, the ability to utilize the compound would confer a great advantage to any microorganism in necrotic tissue, especially if that organism already utilized taurine cleaved from, e.g., taurocholate in the gut.

Lie et al. (31) report that three genera of sulfate reducers, including *D. desulfuricans*, can reduce cysteate and/or isethionate (Fig. 1). The phylogenetic position of the non-sulfate reducer, *B. wadsworthia*, is close to that of the sulfate reducers (Fig. 4); therefore, respiration of sulfonates may be widespread among the sulfate reducers and phylogenetically related genera. Given that *Bilophila* belongs to the normal gut flora (3) and that our isolates are from different sewage works, it seems likely that taurine reduction in respiratory metabolism is a previously unrecognized widespread phenomenon, both in the gut and in anaerobic digestors.

Besides our interest in the novelty of the reaction, the aims of this work were to obtain desulfonation with high specific activity in cell extracts. Whereas the assimilative desulfonation yielded specific activities of about 0.01 mkat/kg of protein (*Clostridium beijerinckii* EV4 [11]), we obtained an increase of about 250-fold in specific rates on moving to dissimilatory reactions of similarly rapidly growing organisms (doubling time, 4.7 h; 2.7 mkat/kg of protein for taurine utilization by *B. wadsworthia* RZATAU).

The disappearance of taurine from cell extracts of *B. wadsworthia* RZATAU depends on the presence of pyruvate; the formation of alanine is detected, and the release of ammonia is accelerated by the presence of NAD^+ (Fig. 6). We presume that the initial reaction is a transamination to form alanine, from which ammonium ion is released by an alanine dehydrogenase. We postulate, without any direct evidence, that the taurine was converted to sulfoacetaldehyde, analogous to the aerobic pathway in, e.g., *Pseudomonas aeruginosa* (24, 27, 40; compare reference 26). The next step in aerobes is the release of sulfite and acetate by a hydrolase (EC 4.4.1.12), which has been purified (25, 40). By analogy, we would then anticipate sulfite reductase catalyzing the terminal electron transfer from an electron transport chain involving energy generation. Whereas we have observed the formation of acetate and detected sulfite reductase, we have as yet found no sulfite and no sulfide in cell extracts. We may have proposed a hypothesis that is too simple or that requires an unidentified sulfite transfer system, but we are continuing to explore the system. Whatever intermediates are involved, if the purpose of the pathway is to deliver sulfite to a sulfite reductase, we should perhaps be careful in referring to the respiration as being novel. It seems more likely that we have observed a novel substrate or set of substrates that permit a sulfite respiration.

If the degradative pathway is closely analogous to that in aerobes, where the amino group of taurine allows the formation of sulfoacetaldehyde that is cleaved hydrolytically, it is easy to understand why taurine, isethionate, and cysteate are the substrates we found; the last two compounds are also readily converted to sulfoacetaldehyde. In contrast, another natural product, methanesulfonate, a major component of the sulfur cycle (2), would be inert under these conditions. We are still trying to detect anaerobic biotransformations of methanesulfonate, which we failed to degrade in other experiments (10, 12).

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