APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1988, p. 2938–2943
0099-2240/88/122938-06$02.00/0

Vol. 54, No. 12

Physiological Characterization of Strain DCB-1, a Unique Dehalogenating Sulfidogenic Bacterium†

TODD O. STEVENS,[1]‡ TIMOTHY G. LINKFIELD,[2]§ AND JAMES M. TIEDJE[1,2]*

Department of Microbiology and Public Health[1] and Department of Crop and Soil Sciences,[2] Michigan State University, East Lansing, Michigan 48824-1325

Received 9 May 1988/Accepted 1 September 1988

Strain DCB-1 is an obligately anaerobic bacterium which carries out the reductive dehalogenation of halobenzoates and was previously known to grow only on pyruvate plus 20% ruminal fluid. When various electron acceptors were supplied, thiosulfate and sulfite were found to stimulate growth. Sulfide was produced from thiosulfate. Cytochrome *c* and desulfoviridin were detected. The mol% G+C was 49 (at the thermal denaturation temperature). Of 55 carbon sources tested, only pyruvate supported growth as the sole carbon source in mineral medium. Lactate, acetate, L- and D-malate, glycerol, and L- and D-arabinose stimulated growth when supplemented with 10% ruminal fluid and 20 mM thiosulfate. In mineral medium, pyruvate was converted to acetate and lactate, with small amounts of succinate and fumarate accumulating transiently. During growth with thiosulfate, all of these products accumulated transiently. Addition of excess hydrogen to pyruvate-grown cultures resulted in diversion of carbon to formate, lactate, and butyrate, which caused a decrease in cell yield. We conclude that strain DCB-1 is a new type of sulfidogenic bacterium.

Microorganisms capable of transforming halogenated aromatic compounds are of interest both as an aid in understanding the persistence of these compounds and for their potential use in removing the compounds from contaminated sites and industrial waste streams. One such organism, designated strain DCB-1, was isolated from an anaerobic 3-chlorobenzoate-degrading sewage sludge enrichment (19) and is the first anaerobe in pure culture capable of aromatic reductive dehalogenation. It is capable of reductively dehalogenating a variety of *meta*-halobenzoates (T. G. Linkfield and J. M. Tiedje, submitted for publication) and of demethoxylating both *meta*- and *para*-methoxybenzoate (3), although it does not degrade the benzoate ring itself. The demethoxylating and dechlorinating activities are not carried out by the same enzyme system (3). The bacterium was described as a very slowly growing, gram-negative, nonsporeforming, obligately anaerobic bacillus which developed an unusual morphological feature (a collar) and had an unusually restricted substrate range (19). Clarified ruminal fluid was the substrate in the original isolation medium; of 28 other substrates tested, only pyruvate was found to support growth (19). Strain DCB-1 also consumed H_2, which inhibited dechlorination at high concentrations (Linkfield and Tiedje, submitted). DCB-1 was recombined with a benzoate-oxidizing organism and a methanogen, both from the original enrichment culture, to produce a syntrophic 3-chlorobenzoate-mineralizing consortium in which 3-chlorobenzoate was the sole carbon and energy source (4). We have further characterized strain DCB-1 in order to better understand its taxonomic position in relation to other anaerobes and its unique metabolism.

* Corresponding author.

† Journal article no. 12643 of the Michigan Agricultural Experiment Station.

‡ Present address: Department of Bacteriology and Biochemistry, University of Idaho, Moscow, ID 83843.

§ Present address: Battelle Columbus Laboratories, Columbus, OH 43201.

MATERIALS AND METHODS

Bacterial strains and culture conditions. Strain DCB-1, *Desulfovibrio* strain PS-1, *Methanospirillum* strain PM-1, and the butyrate oxidizer strain NSF-2 were isolated in this laboratory and previously described (19). *Desulfovibrio* strain DG-2 is described elsewhere (6).

All bacterial growth media were based on the basal salts medium described earlier (19). Media were dispensed into serum bottles or anaerobic culture tubes and sealed with butyl rubber stoppers by the Hungate technique (8). The headspace consisted of 80% N_2–20% CO_2 or 80% H_2–20% CO_2, as specified. Media were reduced by being boiled under a stream of the headspace gas and cooled, after which the reducing agent was added. Initially, the reducing agent was 0.5 mM cysteine hydrochloride plus 0.5 mM $Na_2S \cdot 9H_20$. Because this reductant exhibited some toxicity to the organism (23), 0.5 mM sodium dithionite was used to reduce media in the later experiments on growth yield.

General maintenance medium consisted of the basal salts medium plus sodium pyruvate (0.2%, wt/vol) and clarified ruminal fluid (10%, vol/vol). Prior to transfer, each culture was checked for dechlorination activity by high-pressure liquid chromatography analysis (see below) and for purity by microscopic observation. Measurement of dehalogenation was performed in the same medium but with 800 μM 3-chlorobenzoate. Where described, electron acceptors were added to achieve a 2, 5, 10, or 20 mM concentration. Incubations were stationary, in the dark, and at 37°C. Stock cultures were maintained by transferring with a sterile syringe 10% inoculum into both fresh maintenance medium and dechlorination medium.

When washed cells were required, a volume of the grown culture was transferred to a sterile centrifuge bottle under a stream of O_2-free N_2 or in an anaerobic glove box. The cultures were centrifuged at 16,000 × *g*, the pellets were washed in fresh medium, and the cells were then suspended in additional fresh medium. The cells were maintained under anaerobic conditions throughout this procedure.

Growth studies. Growth of DCB-1 was monitored by measuring the optical density of cultures in 18-mm-diameter

anaerobic culture tubes, using a Turner model 350 spectrophotometer set at 660 nm. Data presented are mean values from five matched tubes used as replicates. Growth on the various substrates was measured in the presence or absence of 10% ruminal fluid, as indicated. No growth was defined as <0.05 and <0.02 optical density units above values for controls with no substrate and with and without thiosulfate, respectively. Carbon sources were used at concentrations of 0.1 to 0.3%. Where indicated, hydrogen additions to the media were made after the cultures were autoclaved by using a Hungate gassing line (8). The inoculum was from 14-day-old cultures.

Experiments to determine metabolic products and cell yield were done by inoculating the washed-cell suspension from stock cultures into 50 ml of mineral medium amended as indicated. Samples were removed periodically with a sterile syringe for determinations of organic acid and protein. The data presented are mean values from four replicates.

Analytical methods. Organic acids were determined by high-pressure liquid chromatography, using an Aminex ion exclusion HPX-87H column (Bio-Rad, Inc., Richmond, Calif.). The mobile phase was either 0.007 or 0.008 N H_2SO_4. Analysis was carried out by using an automated Shimadzu LC-6A system with a UV spectrophotometric detector set at 210 nm. The flow rate was 0.6 ml/min, and the column temperature was either 35 or 60°C. Samples to be analyzed were acidified to pH 3 with 2.5 N H_2SO_4, filtered through a 0.45-μm-pore-size filter, and transferred into clean 1.5-ml autosampler vials for automated injection.

meta-Chlorobenzoate and benzoate were determined by high-pressure liquid chromatography with a Hibar LiChrosorb 10-μm RP-18 column. The mobile phase was a 70:40:13 mixture of water, methanol, and acetic acid, diluted with methanol. The instrument was a Varian 5000 chromatograph coupled to a Hitachi model 100-40 spectrophotometer with an Altex 100-55 flow cell. Detection was by UV absorption at 284 nm with a flow rate of 1.5 ml/min, using a 50:50 mix of the solution described above and methanol. Samples to be analyzed were filtered through a 0.45-μm-pore-size filter before injection.

Hydrogen concentration was measured with a Carle analytical gas chromatograph (model 111) as previously described (18). Sulfide was measured by the Pachmayr method as described by Brock et al. (2).

Protein cytochromes and G+C content. Protein was determined, after washing and alkaline digestion of cells, by the method of Lowry et al. (12). Cytochromes were determined as described by Weston and Knowles (24). Sulfite reductase (desulfoviridin) was identified by the absorption spectrum (25) and by the Postgate procedure (16). For these analyses, bacterial cells were centrifuged anaerobically, suspended in phosphate buffer, pH 7.0, ruptured in a French pressure cell at 9×10^7 Pa, and recentrifuged at 16,300 × *g* for 20 min. The supernatant was analyzed in a double-beam spectrophotometer (model 350; The Perkin-Elmer Corp., Norwalk, Conn.) scanning from 730 to 180 nm on an arbitrary scale of −0.01 to 0.09. Sodium dithionite and $Fe_2(SCN)_6$ were used to reduce and oxidize the cytoplasm to determine the cytochrome spectrum.

Desulfovibrio strains PS-1 and DG-2 served as positive controls for the cytochrome analyses and desulfoviridin tests. *Methanospirillum* strain PM-1 and the butyrate oxidizer strain NSF-2 served as negative controls for the Postgate test.

For determination of moles percent G+C, a 20-ml portion of the cell suspension was mixed with 250 μl of RNase A and the cells were disrupted in a French pressure cell. The lysate was collected in 10 ml of 3× NaCl-EDTA solution (13) and 10 ml of phenol-$CHCl_3$. After centrifugation, the pellet was washed with phenol-$CHCl_3$. The DNA was precipitated with ethanol. Purity was estimated to be 65% by absorbance ratio. Sheared *Escherichia coli* b DNA was also prepared and used as the standard. Samples of DNA from the two test organisms were dialyzed against the same buffer, and thermal denaturation temperatures were determined with a spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Moles percent G+C was estimated by the equation of Marmur and Doty (14).

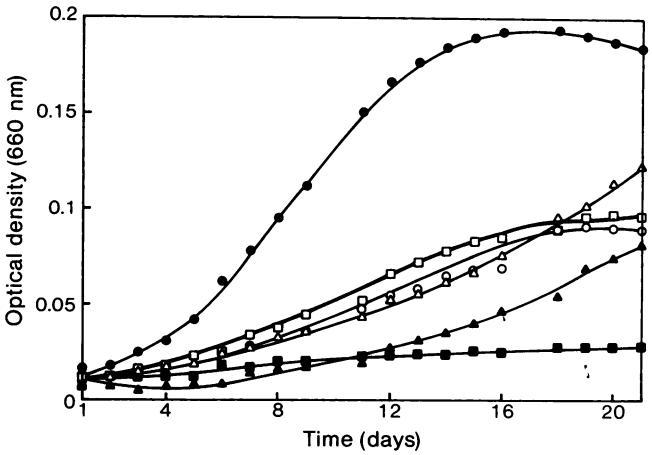

FIG. 1. Growth of DCB-1 in pyruvate medium plus ruminal fluid supplemented with the following electron acceptors: 20 mM $S_2O_3^{2-}$ (●), no electron acceptor added (□), 20 mM SO_4^{2-} (△), 10 mM NO_3^- (○), S^0 (▲), and 20 mM SO_3^{2-} (■).

RESULTS

Effect of various electron acceptors. In an attempt to improve growth and broaden the substrate range of strain DCB-1, various electron acceptors were supplied in medium containing 10% ruminal fluid and pyruvate. Thiosulfate greatly stimulated growth (Fig. 1), but a 20-mM concentration of nitrate, sulfur, or sulfite had no effect or exhibited apparent toxicity. Lower concentrations of sulfuroxy anions were tested (Fig. 2) because of the potential toxicity of some of these anions. In contrast to the rapid growth in the presence of thiosulfate, sulfite now produced slow growth (5 mM) or long lag periods plus slow growth (10 mM). Sulfate was inhibitory to the organism at both 10 and 2 mM; however, slow growth occurred after 7 days in the tubes with 2 mM sulfate, and after 20 days turbidity began to exceed that of the controls (Fig. 1 and 2). Cultures grown with thiosulfate consumed added hydrogen (0.75 kPa/day) more than twice as fast as they did without thiosulfate (0.34 kPa/day). When DCB-1 was incubated with 1 or 2 mM thiosulfate for 14 days, sulfide accumulated to 0.48 and 0.36 mM, respectively, above background. Although the assay could reliably detect sulfide production, it was not quantitative in the presence of thiosulfate.

Cytochromes and G+C content. The cytochrome spectrum of DCB-1 matched that of *Desulfovibrio* strain DG-2 (Fig. 3) and was characteristic of *c*-type cytochromes, with adsorption maxima at 420, 521, and 553 nm (24). The absolute

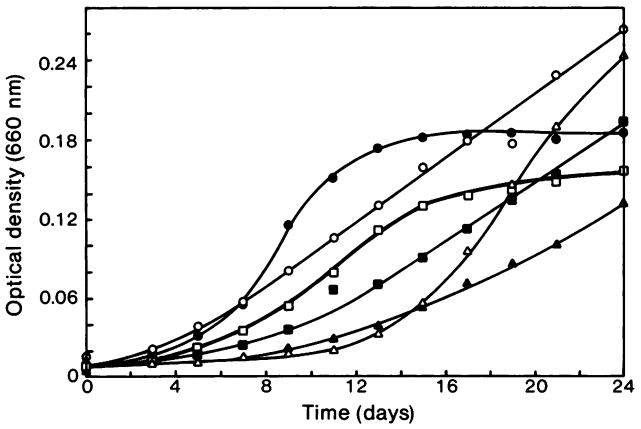

FIG. 2. Effects of various sulfuroxy anion electron acceptors on growth of DCB-1 in pyruvate medium plus 20% ruminal fluid. Symbols: ●, 20 mM $S_2O_3^{2-}$; □, no electron acceptor added; ○, 5 mM SO_3^{2-}; △, 10 mM SO_3^{2-}; ■, 2 mM SO_4^{2-}; ▲, 10 mM SO_4^{2-}.

absorption spectrum showed a peak at 630 nm (Fig. 4), which was also found in *Desulfovibrio* strain DG-2 and corresponded to desulfoviridin (25). Also, *Desulfovibrio* strain PS-1 and strain DCB-1 tested positive for desulfoviridin by showing a strong red fluorescence in alkaline solution (16), whereas two other obligate anaerobes, *Methanospirillum* strain PM-1 and strain NSF-2, tested negative. The mol% G+C was 49.

Substrate range. Various carbon sources were tested to determine whether they supported growth with or without thiosulfate as the electron acceptor in mineral medium plus 10% ruminal fluid. The following substrates and conditions were tested and found not to support significant increases in turbidity: (without thiosulfate) D-gluconic acid, D-glucuronic acid, D-(−)-ribose, ribitol, D-mannitol, *i*-inositol, D-(+)-sorbitol, amylose, inulin, pectin, *N*-acetylglycine, L-ascorbic acid, and malonic acid; (with and without thiosulfate) H_2 plus CO_2, acetate plus N_2, sodium citrate, succinate, choline, formate, D-(+)-mannose, D-glucose, D-(+)-galactose, β-D-(−)-fructose, maltose, sucrose, lactose, D-(+)-xylose, α-L-rhamnose, D-(+)-trehalose, D,L-arabitol, cellobiose, soluble starch, β-alanine, oxalate, valeric acid, caproic acid, isovaleric acid, crotinic acid, butyric acid, isobutyric acid, propionic acid, ethanol, benzoate, phenol, anisole, and D-(+)-fumarate (with thiosulfate only).

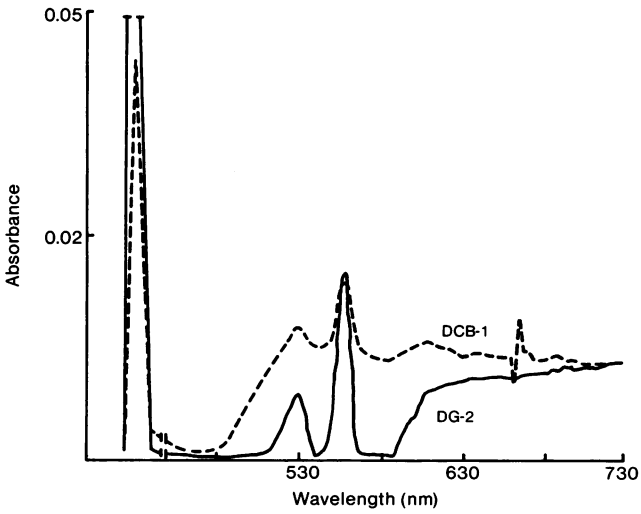

FIG. 3. Reduced-minus-oxidized difference spectrum showing evidence of cytochrome *c* in *Desulfovibrio* strain DG-2 and in DCB-1, which was grown in medium containing pyruvate plus thiosulfate.

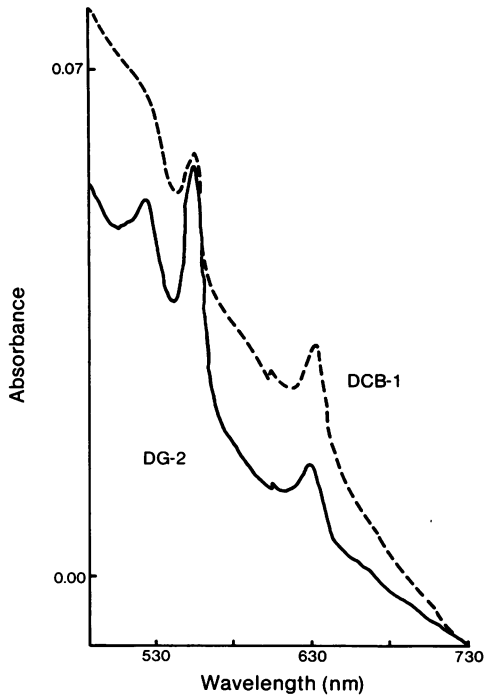

FIG. 4. Absolute spectrum (oxidized) showing evidence of sulfite reductase (desulfoviridin) in *Desulfovibrio* strain DG-2 and in DCB-1, which was grown on medium containing pyruvate plus thiosulfate.

Pyruvate was the only substrate on which the culture could be maintained through several transfers. In medium containing 10% ruminal fluid and 20 mM thiosulfate, growth was stimulated by lactate, acetate plus H_2, L- and D-malate, L- and D-arabinose, and glycerol. Very poor growth was maintained for several transfers with acetate plus H_2, thiosulfate, and 10% ruminal fluid. In the absence of ruminal fluid, only pyruvate supported significant growth with or without an electron acceptor.

Since cysteine was often used as a medium reductant, we determined whether it influenced growth. Washed and suspended cells were used to inoculate media with either cysteine-sulfide or sulfide as the reductant. Growth was not significantly affected by the presence or absence of cysteine, and we concluded that cysteine was not used as a carbon source.

Metabolic products. The time course of metabolic products from pyruvate was studied during growth in mineral medium. Because growth was affected by addition of thiosulfate (positively) and hydrogen (negatively), the effects of these products on metabolic patterns were examined (Fig. 5). With pyruvate alone, acetate and lactate appeared to accumulate continuously (Fig. 5A), whereas succinate accumulated only transiently. When hydrogen was added (Fig. 5B), more acetate and lactate accumulated. Butyrate also accumulated, whereas formate accumulated transiently. When thiosulfate was present (Fig. 5C), acetate as well as succinate and fumarate accumulated only transiently, whereas lactate con-

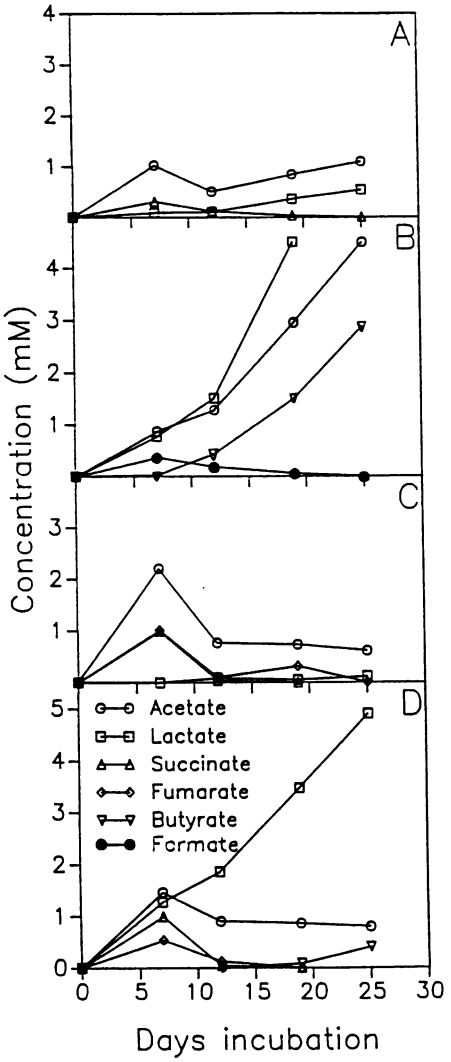

FIG. 5. Products of pyruvate fermentation by DCB-1 grown under the following conditions: (A) pyruvate only, (B) pyruvate plus H_2, (C) pyruvate plus thiosulfate, and (D) pyruvate plus H_2 plus thiosulfate.

centrations remained at low levels. The culture with both thiosulfate and hydrogen (Fig. 5D) showed a fermentation pattern similar to that of the culture with thiosulfate alone, with the exception that some butyrate accumulated, and lactate accumulated to concentrations as high as it did in the treatment containing pyruvate plus hydrogen.

During 25 days of incubation, the pyruvate concentration declined by 11.3 mM in cultures containing pyruvate alone, by 15.1 mM in cultures containing pyruvate and hydrogen, by 10.1 mM in cultures containing pyruvate and thiosulfate, and by 15.1 mM in cultures containing pyruvate, thiosulfate, and hydrogen. The recovery of carbon in organic acids at the end of the experiment was 44% in cultures containing pyruvate alone, 73% in cultures containing pyruvate and hydrogen, 48% in cultures containing pyruvate and thiosulfate, and 39% in cultures containing pyruvate, thiosulfate, and hydrogen.

Cell yields. The molar growth yields obtained under the four sets of conditions listed above are shown in Table 1.

TABLE 1. Cell yields of strain DCB-1 grown on pyruvate and corrected yields with nonproductive diversion of carbon subtracted

Growth medium	Cell yield (g of protein/mol of pyruvate oxidized)	
	Original	Corrected
Pyruvate	6.8 (1.0)[a]	7.6 (1.2)
Pyruvate + H_2	2.4 (0.3)	3.9 (0.2)
Pyruvate + $S_2O_3^{2-}$	10.3 (2.0)	10.7 (2.1)
Pyruvate + H_2 + $S_2O_3^{2-}$	8.4 (1.0)	11.7 (2.4)

[a] Numbers in parentheses are standard deviations.

The corrected values were calculated by assuming that the carbon excreted as lactate was not utilized as a carbon source and by subtracting the number of moles of lactate from the amount of pyruvate utilized. Addition of thiosulfate increased growth yields, whereas addition of hydrogen appeared to decrease them. With the effect of substrate diversion to lactate subtracted, however, the cell yields of cultures containing thiosulfate and hydrogen were similar to those of cultures without added hydrogen. Even with this correction, hydrogen had a negative effect on cell yields during growth on pyruvate only.

DISCUSSION

The stimulation of growth with sulfite and thiosulfate and the production of sulfide from thiosulfate indicate that strain DCB-1 is a sulfidogen. Only thiosulfate and sulfite were able to stimulate growth above that attained without electron acceptors in mineral medium (without ruminal fluid). The slow growth in the presence of sulfate may indicate a low energy yield resulting from the activation energy requirement incurred by formation of adenylphosphosulfate (15). If so, this characteristic would represent a stronger taxonomic link to the *Desulfovibrio*-like organisms than to the *Desulfotomaculum* group, which can conserve this activation energy. Sulfate was a much less desirable electron acceptor than was thiosulfite or sulfite, since growth with sulfate did not exceed that of the controls until 20 days of incubation and then only when the medium was supplemented with ruminal fluid. A further link of DCB-1 to sulfidogenic bacteria was the spectral evidence, which indicated the presence of a *c*-type cytochrome and sulfite reductase (desulfoviridin). In mineral medium with pyruvate as the carbon source, addition of 10 mM thiosulfate caused a 41 to 50% increase in molar growth yield and resulted in only transient production of metabolic products, which accumulated in the absence of an electron acceptor. This finding suggests a shift from a fermentative-type growth on pyruvate to respiration with thiosulfate.

The known substrate range of strain DCB-1 remains quite narrow, as is characteristic of some anaerobic bacteria, although with thiosulfate as the electron acceptor DCB-1 was shown to benefit from the presence of lactate, acetate, L- and D-malate, L- and D-arabinose, and glycerol in complex media. In mineral media, however, only pyruvate could support growth with or without thiosulfate. This requirement for a complex nutrient such as ruminal fluid complicates the interpretation of the growth results. Some unknown growth factors present in ruminal fluid may be required. On the other hand, because of the rich content of fatty acids and other substances in ruminal fluid, it is possible that catabolite

repression interfered with utilization of some of the substrates tested. It is also possible that some yet unknown growth factors would allow growth on more carbon sources. This last possibility is supported by the failure of all substrates except pyruvate and acetate plus H_2 (very poor growth) to support growth through repeated transfers to fresh medium, which suggests gradual dilution of some required nutrient.

Strain DCB-1 has been shown to grow mixotrophically, and the organism fixes CO_2 to provide a large portion of cell carbon while utilizing organic molecules as an energy source (21). That study showed that although little growth occurred with lactate or acetate in mineral media, a mineral medium containing two substrates (lactate and acetate or butyrate and acetate) was capable of supporting growth of DCB-1 with or without thiosulfate. This finding helps to explain why lactate or acetate alone could support growth only in the presence of ruminal fluid; an organic hydrogen acceptor or donor, respectively, was evidently required in addition to thiosulfate.

The patterns of formation of fermentation products (Fig. 5) show that in mineral medium, strain DCB-1 metabolized pyruvate to lactate and acetate. During growth with thiosulfate, thiosulfate was reduced instead of pyruvate and lactate could be reoxidized, which resulted in only a transient accumulation of fermentation products. The transient accumulation of succinate and fumarate, especially when the organism was switched to respiratory growth (Fig. 5C and D), suggests a possible role of these compounds in the energy metabolism of strain DCB-1. It is possible that enzymes of the tricarboxylic acid cycle are present in this organism, as has been described for many sulfate-reducing bacteria (1, 10). The transient accumulation of many products seen in Fig. 5 was probably due to slow metabolic changes incurred by the shift from growth in complex medium to growth in defined medium.

When excess hydrogen was present, lactate was excreted in relatively large amounts, as was butyrate. This diversion of carbon, possibly a mechanism of regulating internal reducing equivalent levels, resulted in a decrease in growth yield (Table 1). When this diverted carbon (lactate) was subtracted from total carbon utilized, the cell yields, at least for growth in thiosulfate, were not significantly different regardless of whether hydrogen was present. During growth without thiosulfate, however, there was an additional depression in cell yield. Addition of hydrogen also resulted in increased acetate excretion, especially in the treatments without thiosulfate (Fig. 5B). This acetate has been shown to be made up mainly of pyruvate carbon; without excess hydrogen, however, a larger portion of this acetate was derived from fixed CO_2 (21). It is likely that this additional diversion of pyruvate carbon was responsible for the portion of cell yield depression not accounted for by diversion to lactate.

Although strain DCB-1 was responsible for the rate-limiting reaction in the 3-chlorobenzoate-degrading enrichment from which it was isolated, it seems unlikely that it plays a key role in food webs of the sewage sludge environment from which that enrichment was derived. A likely niche that DCB-1 may occupy is that of a scavenger of metabolic products from other organisms. It does not seem to be able to utilize larger molecules such as polymers, starches, or sugars as sole carbon sources, but it can use a number of compounds mixotrophically (e.g., with ruminal fluid [21]). These are mostly reduced organic compounds that are commonly excreted by other anaerobic organisms during intermediary metabolism (7, 9, 11) and could be expected in anaerobic environments. Because of its very low growth rate (19), DCB-1 may not compete well for intermediary metabolic compounds. Perhaps it is able to use amounts of carbon too low for other organisms to take up, to use more unusual electron acceptors, or to exploit a more refractory pool of fatty acids that may be present in anaerobic environments (22).

DCB-1 grows as a member of a syntrophic consortium, which may gain energy by the dechlorination of 3-chlorobenzoate (5). Since chlorobenzoate is unlikely to be the original substrate of an energy-conserving pathway, this characteristic may indicate an ability to derive energy from some other complex but naturally occurring molecule. It is possible that strain DCB-1 has adapted to a primarily syntrophic niche which laboratory conditions approximate only poorly.

Strain DCB-1 appears to be closely related to the sulfate-reducing bacteria in that its growth is stimulated by oxidized sulfur compounds as electron acceptors and it has a similarly restricted substrate range. The presence of cytochrome *c* and desulfoviridin in DCB-1 and the mixotrophic metabolism of the bacterium, previously described in some sulfate-reducing bacteria (17, 20), also seem to indicate a close relationship. Its mol% G+C of 49 is lower than that of most sulfate-reducing bacteria but is in the range reported (26) for *Desulfuromonas acetoxidans* (50 to 52%), *Desulfovibrio salexigens* (46.1%), *Desulfovibrio saprorovans* (52.7%), and *Desulfosarcina* spp. (51.2%). With respect to other major characteristics, however, these organisms are dissimilar to DCB-1. Furthermore, because of the poor growth of DCB-1 in the presence of sulfate, the unique collar surrounding every cell (19), and the organism's excretion of acetate formed from CO_2 (21) and unique dehalogenating ability, we have decided not to name this bacterium until molecular taxonomic studies have been completed.

ACKNOWLEDGMENTS

We thank Joyce Wildenthal for technical assistance and Bill Mohn for comments on the manuscript. We also thank John L. Johnson for determining the moles percent G+C.

This work was supported by grants from the U.S. Air Force Engineering & Services Laboratory, the U.S. Department of Agriculture, and the Michigan Research Excellence Fund.

LITERATURE CITED

1. **Brandis-Heep, A., N. A. Gebhardt, R. K. Thauer, F. Widdel, and N. Pfennig.** 1983. Anaerobic acetate oxidation to CO_2 by *Desulfobacter postgatei*. 1. Demonstration of all enzymes required for the operation of the citric acid cycle. Arch. Microbiol. **136:** 222–229.
2. **Brock, T. D., M. L. Brock, T. L. Bott, and M. R. Edwards.** 1971. Microbial life at 90°C: the sulfur bacteria of Boulder Spring. J. Bacteriol. **107:**303–314.
3. **DeWeerd, K. A., J. M. Suflita, T. G. Linkfield, J. M. Tiedje, and P. H. Pritchard.** 1986. The relationship between reductive dehalogenation and other aryl substituent removal reactions catalyzed by anaerobes. FEMS Microbiol. Ecol. **38:**331–339.
4. **Dolfing, J., and J. M. Tiedje.** 1987. Hydrogen cycling in a three-tiered food web growing on the methanogenic conversion of 3-chlorobenzoate. FEMS Microbiol. Ecol. **38:**293–298.
5. **Dolfing, J., and J. M. Tiedje.** 1987. Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. Arch. Microbiol. **149:**102–105.
6. **Dwyer, D. F., and J. M. Tiedje.** 1986. Metabolism of polyethylene glycol by two anaerobic bacteria, *Desulfovibrio desulfuricans* and a *Bacteroides* sp. Appl. Environ. Microbiol. **52:**852–856.
7. **Hobson, P. N., S. Bousfield, and R. Summers.** 1974. Anaerobic

digestion of organic matter. Crit. Rev. Environ. Control **4**:131–191.
8. **Hungate, R. E.** 1968. A roll rube method for cultivation of strict anaerobes, p. 117–132. *In* J. R. Norris and D. W. Ribbons (ed.), Advances in microbiology, vol. 3B. Academic Press, Inc., New York.
9. **Hungate, R. E.** 1975. The rumen microbial ecosystem. Annu. Rev. Ecol. Syst. **6**:39–66.
10. **Lewis, A. J., and J. D. A. Miller.** 1977. The tricarboxylic acid pathway in *Desulfovibrio*. Can. J. Microbiol. **23**:916–921.
11. **Lovley, D. R., and M. J. Klug.** 1982. Intermediary metabolism of organic matter in the sediments of a eutrophic lake. Appl. Environ. Microbiol. **43**:552–560.
12. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. **3**:208–218.
14. **Marmur, J., and P. Doty.** 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. Mol. Biol. **5**:109–118.
15. **Peck, H. D., Jr., and J. LeGall.** 1982. Biochemistry of dissimilatory sulphate reduction. Phil. Trans. R. Soc. London Ser. B **298**:443–466.
16. **Postgate, J.** 1959. A diagnostic reaction of *Desulfovibrio desulphuricans*. Nature (London) **163**:481–482.
17. **Rittenberg, S. C.** 1969. The roles of exogenous organic matter in the physiology of chemolithotrophic bacteria. Adv. Microbiol. Physiol. **3**:159–196.
18. **Robinson, J. A., and J. M. Tiedje.** 1982. Kinetics of hydrogen consumption by rumen fluid, anaerobic digestor sludge, and sediment. Appl. Environ. Microbiol. **44**:1374–1384.
19. **Shelton, D. R., and J. M. Tiedje.** 1984. Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic acid. Appl. Environ. Microbiol. **48**: 840–848.
20. **Sorokin, Y. I.** 1966. Sources of energy and carbon for biosynthesis in sulfate-reducing bacteria. Mikrobiologiya **35**:761–766.
21. **Stevens, T. O., and J. M. Tiedje.** 1988. Carbon dioxide fixation and mixotrophic metabolism by strain DCB-1, a dehalogenating anaerobic bacterium. Appl. Environ. Microbiol. **54**:2944–2948.
22. **Thompson, L. A., and D. B. Nedwell.** 1985. Existence of different pools of fatty acids in anaerobic model ecosystems and their availability to microbial metabolism. FEMS Microbiol. Ecol. **31**:141–146.
23. **Tiedje, J. M., and T. O. Stevens.** 1988. The ecology of an anaerobic dechlorinating consortium, p. 3–14. *In* G. S. Omenn, R. Colwell, A. Chakrabarty, M. Levin, and P. McCarty (ed.), Environmental biotechnology; reducing risks from environmental chemicals. Plenum Publishing Corp., New York.
24. **Weston, J. A., and C. J. Knowles.** 1973. A soluble CO-binding c-type cytochrome from the marine bacterium *Beneckea natriegens*. Biochim. Biophys. Acta **305**:11–18.
25. **Widdel, F., G.-W. Kohring, and F. Mayer.** 1983. Studies on the dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonemia magnum* sp. nov. Arch. Microbiol. **134**:286–294.
26. **Widdel, F., and N. Pfennig.** 1984. Dissimilatory sulfate- or sulfur-reducing bacteria, p. 663–679. *In* N. R. Krieg and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The William and Wilkins Co., Baltimore.