APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1980, p. 626-632
0099-2240/80/09-0626/07$02.00/0
Vol. 40, No. 3

Propionate-Degrading Bacterium, *Syntrophobacter wolinii* sp. nov. gen. nov., from Methanogenic Ecosystems

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A new genus and species of a nonmotile gram-negative rod, *Syntrophobacter wolinii*, is the first bacterium described which degrades propionate only in coculture with an H_2-using organism and in the absence of light or exogenous electron acceptors such as O_2, sulfate, or nitrate. It was isolated from methanogenic enrichments from an anaerobic municipal sewage digestor, using anaerobic roll tubes containing a medium with propionate as the energy source in association with an H_2-using, sulfate-reducing *Desulfovibrio* sp. which cannot utilize fatty acids other than formate. *S. wolinii* produced acetate and, presumably, CO_2 and H_2 (or formate) from propionate. In media without sulfate and with *Methanospirillum hungatei*, a methanogen that uses only H_2-CO_2 or formate as an energy source, acetate, methane, and, presumably, CO_2 were produced from propionate and only small amounts of *Desulfovibrio* sp. were present. Isolation in coculture with the methanogen was not successful. *S. wolinii* does not use other saturated fatty acids as energy sources.

Modern concepts of the complete anaerobic degradation of organic matter and methanogenesis in ecosystems without light and low in sulfate, nitrate, or similar electron acceptors indicate that the process is carried out mainly by three general metabolic groups of bacteria with strong metabolic interactions between groups (5, 7, 9, 11, 18; D. R. Boone and P. H. Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q82, p. 208). A complex of fermentative bacteria ferments the primary substrates such as cellulose, proteins, and lipids to products such as saturated fatty acids, H_2, and CO_2. The terminal metabolic group includes diverse species of methanogens per se (1), and the two chief and essential functions of this group are to catabolize the large amount of acetate produced by the primary and secondary microbial groups to methane and CO_2 and to reduce CO_2 to methane, using the H_2 produced by the other groups as the electron donor (equation C, Table 1).

The second metabolic group (obligate proton-reducing acetogenic bacteria) is a complex of species involved in: β-oxidation of fatty acids of even numbered carbons to acetate and H_2 (equation A, Table 1) and of fatty acids of odd-numbered carbons to acetate, propionate, and H_2; decarboxylation of propionate to acetate, CO_2, and H_2 (equation B, Table 1); and, possibly, other reactions such as degradation of certain aromatic acids (9). This second group is much less well documented than the primary group and the methanogens (5, 6). The pioneering work of Stadtman and Barker (15), using "highly purified" cultures, resulted in the naming of two methanogenic bacteria, *Methanobacterium suboxidans*, which β-oxidized short-chain fatty acids, and *Methanobacterium propionicum*, which oxidatively decarboxylated propionic acid. However, rather than producing H_2, these two species were thought to utilize the electrons generated in the oxidations to reduce CO_2 to methane (equations A + C and B + C, Table 1). They were not purported to be pure and were later lost. In 1967, the discovery that *Methanobacillus omelianskii* was composed of a bacterium that grew and produced acetate and H_2 from ethanol only in syntrophic association with an H_2-using bacterium such as a methanogen (7) led to the idea that propionate and longer-chained fatty acids were catabolized by similar syntrophic associations. This idea is now supported by a considerable body of evidence (5, 6, 11, 18; Boone and Smith, submitted); however, the direct demonstration (via isolation in coculture with an H_2-using single species) of a nonmethanogenic species that degrades any of the fatty acids has only recently been accomplished (13).

The anaerobic bacterium β-oxidizing the normal monocarboxylic, saturated, C_4 to C_8 fatty acids, with acetate, or acetate and propionate, and H_2 being the products (equation B, Table 1), was isolated in coculture with a single H_2-utilizing bacterium, either a methanogen (equations A + C, Table 1) or a *Desulfovibrio* sp. (13;

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TABLE 1. *Equations and free-energy changes for reactions involving anaerobic oxidation of butyrate and propionate to acetate or acetate and HCO_3^-, with protons, HCO_3^-, or SO_4^{2-} serving as electron acceptor, by butyrate- or propionate-catabolizing bacteria in pure culture or in coculture with H_2-utilizing methanogens or Desulfovibrio sp.*[a]

Equation	$\Delta G^{0'}$ (kJ/reaction)
I. Proton-reducing (H_2-producing) acetogenic bacteria	
A. $CH_3CH_2CH_2COO^- + 2H_2O \rightleftharpoons 2CH_3COO^- + 2H_2 + H^+$	+48.1
B. $CH_3CH_2COO^- + 3H_2O \rightleftharpoons CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1
II. H_2-using methanogens and desulfovibrios	
C. $4H_2 + HCO_3^- + H^+ \rightleftharpoons CH_4 + 3H_2O$	−135.6
D. $4H_2 + SO_4^{=} + H^+ \rightleftharpoons HS^- + 4H_2O$	−151.9
III. Cocultures of I and II	
A + C $2CH_3CH_2CH_2COO^- + HCO_3^- + H_2O \rightleftharpoons 4CH_3COO^- + H^+ + CH_4$	−39.4
A + D $2CH_3CH_2CH_2COO^- + SO_4^{=} \rightleftharpoons 4CH_3COO^- + H^+ + HS^-$	−55.7
B + C $4CH_3CH_2COO^- + 12H_2 \rightleftharpoons 4CH_3COO^- + HCO_3^- + H^+ + 3CH_4$	−102.4
B + D $4CH_3CH_2COO^- + 3SO_4^{=} \rightleftharpoons 4CH_3COO^- + 4HCO_3^- + H^+ 3HS^-$	−151.3

[a] H_2 and CH_4 in the gaseous state; all other elements in aqueous solution at 1 mol of activity per kg; 25°C (16).

equations A + D, Table 1). Isolation of the butyrate-oxidizing bacterium in coculture was difficult because of the thermodynamics of the reactions carried out and the necessity for an extremely low partial pressure of H_2 via coculture with the H_2-utilizing bacterium, was previously discussed (13, 18). By these criteria the propionate degrader would be even more difficult to isolate in coculture (equations B, B + C, and B + D, Table 1).

We now report the isolation in coculture with an H_2-utilizing *Desulfovibrio* sp. of a bacterium, *Syntrophobacter wolinii* gen. nov., sp. nov., which degrades propionate to acetate and, presumably, CO_2 and H_2.

MATERIALS AND METHODS

Organisms. *Methanospirillum hungatei* (8) strain DSM 864 (German Culture Collection) was supplied by Ferry and Wolfe (University of Illinois, Urbana). *Desulfovibrio* strain G11 (13), a hydrogen-utilizing sulfate reducer unable to dissimilate fatty acids other than formate, was from our culture collection.

Media and growth conditions. Anaerobic techniques were essentially those of Hungate (10) as modified by Bryant (4); exceptions were fermentation balance experiments and liquid cultures of *M. hungatei* which were grown in special 18- by 150-mm tubes modified for use with black rubber stoppers and aluminum serum cap closures to withstand gas pressures of 2 atm, using the anaerobic techniques of Balch, Wolfe, and co-workers (1, 2). The incubation temperature was 35°C except for some stock cultures, which were maintained at 30°C or at room temperature after growth.

Basal medium (13) and dilution solution contained 5% rumen fluid, minerals with little SO_4^{2-}, B vitamins, and, usually, an 80% N_2–20% CO_2 gas phase. A separately sterilized 7% (vol/vol) solution of 5% $NaHCO_3$ and 2% (vol/vol) reducing solution (1.25% each $Na_2S \cdot 9H_2O$ and cysteine-HCl) were added after the medium was sterilized (13).

Stock cultures of *M. hungatei* and *Desulfovibrio* sp. were maintained in slants of basal medium modified to contain 1% Ionagar no. 2 (Colabs, Chicago Heights, Ill.), 30% (vol/vol) rumen fluid, a 10% (vol/vol) solution of 5% $NaHCO_3$, 20 mM each sodium formate and Na_2SO_4, and 5 mM sodium acetate, with a gas phase of 1:1 H_2-CO_2. The medium was added in 4-ml amounts to rubber-stoppered tubes (13 by 100 mm), which were inoculated by stabbing; they were incubated until growth occurred and were transferred at about 2-week intervals.

Stock coculture slants of *S. wolinii-Desulfovibrio* sp. were added to tubes which were inoculated in the same manner as above but which contained basal medium plus 20 mM each sodium propionate and Na_2SO_4 and 1% Ionagar. Stock cocultures of *S. wolinii-Desulfovibrio* sp.-*M. hungatei* were made using the same medium except that sulfate was deleted.

Liquid cultures of *Desulfovibrio* sp. were added (with shaking) in 5-ml amounts to rubber-stoppered tubes (18 by 150 mm) and inoculated with 0.1 ml of culture. The medium used was basal medium plus 5 mM sodium acetate and 20 mM Na_2SO_4 with 20% CO_2–80% H_2 as the gas phase. The gas phase was replenished as necessary during growth (7). Liquid cultures of *M. hungatei* were the same except sulfate was deleted and a 10% CO_2–90% H_2 gas phase was added at 2-atm pressure (1, 2).

Liquid cocultures of *S. wolinii* and *Desulfovibrio* sp. were maintained in basal medium plus 20 mM each Na_2SO_4 and sodium propionate and were routinely maintained without shaking by weekly transfer of 5 ml to 5 ml of fresh medium in rubber-stoppered tubes (18 by 150 mm). Cocultures containing *S. wolinii*, *M. hungatei*, and *Desulfovibrio* sp. were maintained in the same manner except that sulfate was deleted from the medium.

Enrichments. Propionate-degrading enrichments were developed from primary anaerobic digestor sludge of the Urbana-Champaign Sanitary District by diluting the sludge 1:20 with basal medium and adding 1 ml to 9 ml of basal medium with 20 mM propionate

and only 0.05% (wt/vol) sulfide as the reducing agent and to a control medium without propionate. After 2 weeks of incubation, the tube with propionate showed growth, measured by optical density, and visible gas production greater than those of the control. When growth stopped, 5 ml was transferred to 5 ml of fresh medium. Henceforth, 50% transfers to fresh media were made when it was judged from the optical density that propionate degradation was nearly complete. Later, routine 50% transfers were made weekly.

Isolation with H_2-utilizing lawns. For isolation of *S. wolinii* in coculture with *Desulfovibrio* sp., serial 10-fold dilutions of the propionate enrichment were made in tubes with 4 ml of basal medium plus 0.5 ml of actively growing *Desulfovibrio* sp. culture. Triplicate rubber-stoppered roll tubes (18 by 150 mm) containing basal medium with 20 mM sulfate plus or minus 20 mM propionate and 2% Ionagar no. 2 were inoculated with 0.1 ml of dilutions giving final inocula of 1,000, 100, 10, and 1 nl. For isolation in coculture with *M. hungatii*, 0.5 ml of actively growing *M. hungatei* culture replaced 0.5 ml of *Desulfovibrio* sp. culture, and sulfate was omitted from the roll tubes.

Cultures were checked for purity by examination of wet mounts, using phase microscopy, and by visual and microscopic examination after inoculation and during incubation for 2 to 3 months in fluid thioglycolate medium (Oxoid). Neither *M. hungatei* nor *S. wolinii* grew in this medium, and *Desulfovibrio* sp. showed barely perceptible growth, which was detected with the microscope.

Analytical methods. Optical density for growth estimation of cultures was determined in culture tubes (18 by 150 mm), using a Bausch and Lomb Spectronic 70 spectrometer set at 600 nm. Volumes of tubes were determined by difference in weight between the stoppered tube full of water and the dry tube plus stopper. When gas pressure in the tubes was greater than atmospheric, the excess gas was measured with a syringe (9). Gas was analyzed for H_2, CH_4, and CO_2 by gas chromatography (13). Sulfide was determined by the method of Truper and Schlegel (17), and fermentation acids were determined by the butylester method of Salanitro and Muirhead (14).

RESULTS

Isolation of *S. wolinii* in coculture. After several transfers the enrichment became stabilized with growth and visible gas production. Visual examination of wet mounts with the phase-contrast microscope showed large numbers of motile cells microscopically identical to *M. hungatei* and large filamentous cells like the acetate-degrading *Methanobacterium soehngenii* (3). Smaller numbers of various morphotypes, including some similar to *S. wolinii*, were seen, but it was not possible to detect which one was the propionate oxidizer.

Using a propionate enrichment as inoculum, roll tubes with *Desulfovibrio* sp. lawns were inoculated. After 2 to 3 weeks of incubation, large colonies formed with high inocula (1,000 and 100 nl) of tubes with propionate; these colonies were not present in tubes without propionate. Microscopic examination of these colonies revealed short motile, rod-shaped cells with tapered ends in chains, but no *Desulfovibrio* sp. These bacteria were probably the propionate-oxidizing sulfate reducers, *Desulfobulbus* spp., described by Widdel and Pfennig (personal communication); one of us (M.P.B.) had isolated these bacteria in low numbers from methanogenic propionate enrichments after the original isolation of other strains by Widdel and Pfennig. Continued incubation of roll tubes yielded large (>1 mm), isolated, dark-centered colonies with lower inocula (10 and 1 nl) after 6 weeks of incubation. The colonies were present in tubes with propionate, but absent in controls. They were picked to slants with 20 mM propionate and 20 mM sulfate, incubated for 3 weeks, and examined microscopically. To several isolates 1 ml of liquid medium was added. After overnight incubation, 0.2 ml from each of these cultures was transferred to liquid media with 20 mM sulfate plus or minus 20 mM propionate. After 2 weeks of incubation three isolates showed higher optical densities in tubes containing propionate than in control tubes. Each of these was similar morphologically; a large, short rod, often in pairs and sometimes in chains or filaments, as well as a *Vibrio* sp. morphologically identical to the *Desulfovibrio* strain (Fig. 1) were present. One of the cocultures was chosen for further study. The roll tube isolation procedure was repeated with the coculture as inoculum to insure a pure coculture of a single strain of propionate oxidizer with *Desulfovibrio* sp. After 5 weeks of incubation, only colonies similar to those described above were seen, except that they were smaller in tubes with a larger inoculum and, therefore, more colonies. Well-isolated large colonies from tubes with less inocula were picked to slants of propionate-sulfate medium. They were all the same morphologically, and one of these (strain DB) was maintained for further studies.

The isolation of this propionate-degrading bacterium in coculture with *M. hungatei* was attempted. Slants with 20 mM propionate and 0.5 mM sulfate were stab-inoculated with the *S. wolinii-Desulfovibrio* sp. coculture and with *M. hungatei*. After 2 weeks, gas splits were seen in the agar, and the culture was transferred to slants with 20 mM propionate and no sulfate. After incubation, microscopic examination revealed large numbers of *M. hungatei* and the propionate oxidizer and only small numbers of *Desulfovibrio* sp. The culture was inoculated into liquid medium with 20 mM propionate, and isolation was attempted by using roll tubes with *M. hungatei* lawns. Very small colonies of *De-*

sulfovibrio sp. developed only in tubes with the largest inoculum; no other colonies developed even after 3 months of incubation. This procedure was repeated three times, and isolation of a propionate oxidizer directly from the enrichment culture was also attempted by using roll tubes with *M. hungatei* lawns, but all attempts were unsuccessful. After 3 months of incubation of roll tubes, the resorufin in about half of the tubes had turned pink, indicating exposure to oxygen.

Morphology. *S. wolinii* was a gram-negative rod which occurred in pairs with many single cells and short chains, and also with filaments (Fig. 1). Cells were highly refractile, 0.6 to 1.0 μm wide and 1.0 to 4.5 μm long, with filaments up to 35 μm long. No spores were observed. The organism was not seen to be motile, and no flagella were observed with electron microscopy of cells stained with uranyl acetate or phosphotungstic acid, although flagella of the *Desulfovibrio* sp. were readily observed. Thin-section electron micrographs confirmed the gram-negative nature of the cell wall.

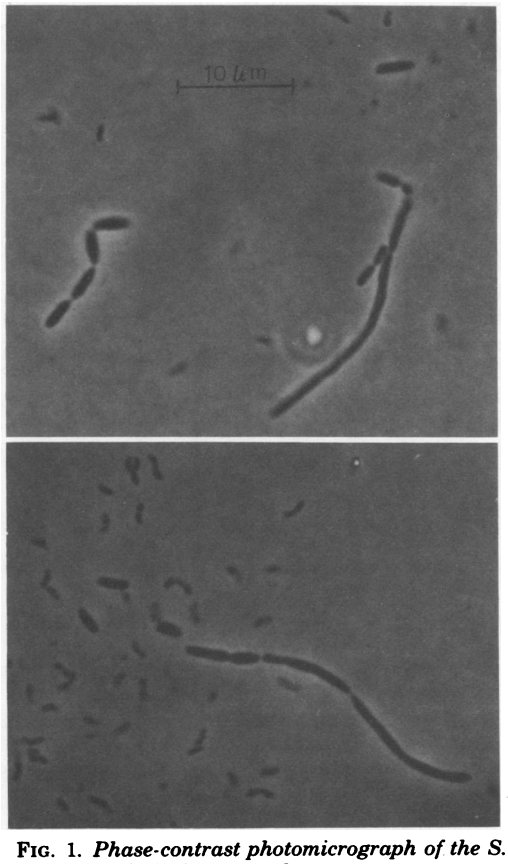

FIG. 1. *Phase-contrast photomicrograph of the S. wolinii-Desulfovibrio sp. coculture.*

Growth rates. Growth rates of the *S. wolinii-Desulfovibrio* sp. coculture were determined using basal medium with 20 mM propionate and 20 mM sulfate and an 8% inoculum of the coculture (Fig. 2). Four determinations based on the steeper part of the curves were averaged, to give a doubling time of 87 ± 7 h. The growth rate of the *S. wolinii-M. hungatei* coculture (with *Desulfovibrio* sp. present) was determined in triplicate tubes, using media with 20 mM propionate but without sulfate. A doubling time of 161 ± 18 h was found.

Sulfide inhibition. Concentrations of 5 and 10 mM Na_2S added to a 15 mM propionate–5 mM Na_2SO_4 coculture of *S. wolinii-Desulfovibrio* sp. inhibited propionate degradation by about 23 and 51% when analyzed after 1 month of incubation, whereas 15 mM sodium acetate or NaCl inhibited it by only 13 and 6%, respectively. Growth was similarly affected. Whether the main effect of sulfide was on *S. wolinii* or *Desulfovibrio* sp. is not presently determinable, but this and other strains of *Desulfovibrio* sp. rapidly utilized at least 50 mM lactate (equivalent to production of 25 mM sulfide), suggesting that the main effect of sulfide was on *S. wolinii*.

Substrate specificity. The *S. wolinii-Desulfovibrio* sp. coculture grew only when propionate and sulfate were present in the medium and other fatty acids did not support growth (Table 2). In media containing substrates such as lactate plus sulfate or pyruvate, which are utilized directly by the *Desulfovibrio* sp., the organism grew so rapidly that it was not possible to detect the possibility of growth by *S. wolinii*. Neither organism grew in medium containing propionate and sulfate if tubes of medium were opened to allow brief exposure to air. In stab cultures,

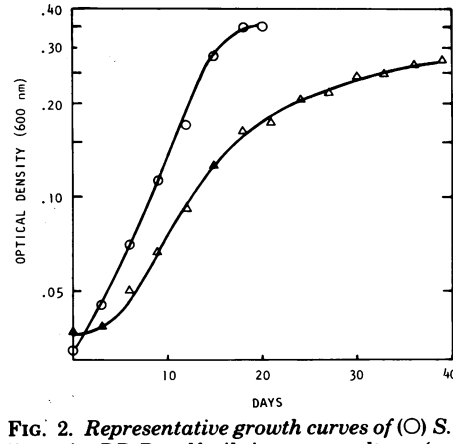

FIG. 2. *Representative growth curves of* (○) *S. wolinii strain DB-Desulfovibrio sp., coculture (propionate plus sulfate) and* (△) *S. wolinii-M. hungatei-Desulfovibrio sp. coculture (propionate without sulfate).*

growth was only evident deep in the tubes well below the point where resorufin was pink; the same was true with sulfate deleted from the medium and *M. hungatei* added to the culture, except that gas was then produced in the deeper part of the stab culture. This suggests that *S. wolinii* is a strict anaerobe.

Fermentation products. The *S. wolinii-Desulfovibrio* sp. coculture grown on sulfate and propionate produced acetate, sulfide, and, presumably, CO_2, and the *S. wolinii-M. hungatei-Desulfovibrio* sp. coculture without sulfate added to the medium produced acetate, methane, and, presumably, CO_2 (Table 3). No organic acids such as pyruvate, lactate, or succinate were detected. The amounts of products are in accord with the stoichiometries of equations B + D and B + C (Table 1).

TABLE 2. *Energy sources for the S. wolinii strain DB-Desulfovibrio strain G11 coculture or for the Desulfovibrio strain alone*[a]

Inoculum	Energy Source	Growth (OD)	Sulfide (mM)
DB + G11	15 mM propionate	0.24	14.4
DB + G11	None	0.07	4.5
DB + G11	15 mM propionate (minus sulfate)	0.07	5.0
DB + G11	25 mM acetate	0.07	5.3
DB + G11	10 mM butyrate	0.08	5.6
DB + G11	10 mM caproate	0.08	3.9
DB + G11	1 mM palmitate	ND	4.6
G11	15 mM propionate	0.08	4.0
G11	None	0.07	4.0
G11	20 mM formate	0.14	8.9
G11	Hydrogen	0.25	15.2
G11	Hydrogen (minus sulfate)	0.07	4.5

[a] Data represent results from duplicate tubes of medium containing 12.5 mM Na_2SO_4 except as indicated and incubated for 31 days before analysis. Inoculum contained sulfate. OD, Optical density; ND, not determined.

DISCUSSION

Saturated fatty acids are quantitatively the most important extracellular organic intermediates involved in the methanogenic fermentation of organic matter; and, with the exception of acetate, which is directly fermented by methanogens, propionate is often quantitatively the most important fatty acid intermediate (11, 12, 18). Although much of the recent research strongly suggests that propionate is degraded via syntrophic association(s) of acetate, CO_2, and H_2-producing bacteria and H_2-utilizing methanogens (5, 6, 11, 18; Boone and Smith, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q82, p. 208), the present study directly documents a species of bacterium which, in a coculture containing only it and an H_2-utilizing sulfate reducer, carried out the anaerobic degradation of propionate and sulfate to acetate, sulfide, and, presumably, CO_2. Furthermore, when *M. hungatei* was added to the coculture and sulfate was deleted from the medium, methane replaced sulfide as the electron sink product.

Our failure to obtain colonies of *S. wolinii* in coculture with *M. hungatei* alone, and thus to obtain a coculture not containing some cells of *Desulfovibrio* sp., may be due to the extremely slow growth rate of the culture (doubling time, 161 h) when CO_2 reduction to methane was the electron sink product in propionate catabolism as compared with the sulfate reduction to sulfide when *Desulfovibrio* sp. was present (doubling time, 87 h). Because H_2-utilizing reactions occur near equilibrium, the more favorable free-energy change of sulfide production may allow the *Desulfovibrio* sp. to oxidize H_2 with enzyme systems having greater affinities for H_2 than do hydrogenases of methanogens. In addition to the

TABLE 3. *Fermentation products formed by the S. volinii strain DB-Desulfovibrio strain G11 coculture growing on medium with 15 mM each propionate and sulfate and by the S. wolinii strain DB-M. hungatei strain JF1-Desulfovibrio strain G11 coculture grown on the same medium minus sulfate*[a]

Coculture	μmol/ml					Recovery (%) of:	
	Propionate degraded	Acetate produced	CO_2 produced[b]	Sulfide produced	Methane produced	Carbon	Hydrogen
DB + G11	13.4	12.0	13.4	10.6	ND[c]	93.0	96.4
DB + JF1 + G11	12.8	11.6	3.2	ND	9.2	92.7	92.9

[a] Data are averages of three fermentation balance experiments with each coculture. Incubation time was 35 days for strain DB plus G11 and 49 days for strains DB plus JF1 and G11. Products in the cultures without propionate added, due to carry-over in the inoculum, were subtracted from those with propionate.

[b] Calculated according to the amount of propionate degraded and the stoichiometry shown in Table 1, equations B + C or B + D.

[c] ND, Not determined.

faster growth rate of the sulfide-generating coculture, sulfide produced as such may play a role in maintaining anaerobiosis in roll tubes during the long incubation time required.

Further attempts to coculture *S. wolinii* with an H_2-using methanogen alone should be attempted with *M. hungatei* or other H_2-using methanogens with anaerobic techniques which maintain the anaerobic conditions for longer periods of time. Pure cultures of *S. wolinii* should be attempted after the organism is obtained in pure coculture with a methanogen with more limited energy sources (as compared with *Desulfovibrio* sp.) so that the possibility of energy sources utilizable by *S. wolinii* alone can be studied effectively.

S. wolinii represents a new genus of bacterium because no other pure or known two-species cocultures of bacteria have been named which anaerobically degrade propionic acid in the absence of a light or electron acceptor such as sulfate or nitrate. From our experience with enrichments it seems probable that the gram-positive, motile cells of *M. propionicum* from mud of San Francisco Bay (15) represent an unknown species of H_2-using methanogen. The mud could have contained *S. wolinii* which would be difficult to detect.

The type species of the genus *S. wolinii* is named in honor of M. J. Wolin, who first suggested that there might be bacteria in nature that would grow with fatty acids and possibly other energy sources only in the presence of H_2-using organisms such as methanogens. He further suggested that such associations might be isolated by using lawns of methanogens similar to those used in this and a previous study (13).

Syntrophobacter wolinii gen. nov. sp. nov., syn.tro.pho.bac´.ter, Gr. adj. *syn* together with; Gr. n. *trophos* one who feeds; M.L. n., *bacter*, masc. equivalent of Gr. neut. n. *bacterion* small staff, rod; M.L. masc. n. *Syntrophobacter* rod which feeds together with (another species); wolin´.i.i, M.L. gen. n. *wolinii* of Wolin.

Cells are gram-negative rods occurring singly and in pairs with some short chains and filaments. The cell width is 0.6 to 1 μm, with filaments up to 35 μm long. The width of a cell is sometimes irregular, especially in filaments. Cells are highly refractile, but no endospores have been observed. Nonmotile. Strictly anaerobic. Mesophilic.

Surface colonies in roll tubes of *S. wolinii-Desulfovibrio* sp. cocultures are black-centered, convex, and circular, with entire edges. Colonies form in 3 to 5 weeks and may reach 2 to 3 mm in diameter. Subsurface colonies are lenticular.

Energy-yielding metabolism involves oxidation of propionate, but not other fatty acids, with the production of acetate and, presumably, H_2 and CO_2. Growth occurs only when H_2 is constantly removed by the action of H_2-utilizing bacteria. Sulfate does not serve as an electron acceptor in the energy metabolism, and no growth occurs in thioglycolate medium. Thus, glucose or materials present in the large amount of yeast extract and tryptic digest of casein added do not support growth.

ACKNOWLEDGMENTS

We thank David Odelson for help in the quantitation of acids and for taking the photomicrographs. Also, our thanks go to Thomas MacAdoo, Virginia Polytechnic Institute and State University, Blacksburg, for help in naming the organism. Arthur Wellinger constructed the pressurized gassing system used, and Paul Smith, University of Florida, Gainesville, confirmed the gram-negative characters with thin-section electron micrographs.

This research was supported by National Science Foundation grant PCM 77-17542 and by the Agricultural Experiment Station of the University of Illinois.

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