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Growth of Syntrophic Propionate-Oxidizing Bacteria with Fumarate in the Absence of Methanogenic Bacteria

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Oxidation of succinate to fumarate is an energetically difficult step in the biochemical pathway of propionate oxidation by syntrophic methanogenic cultures. Therefore, the effect of fumarate on propionate oxidation by two different propionate-oxidizing cultures was investigated. When the methanogens in a newly enriched propionate-oxidizing methanogenic culture were inhibited by bromoethanesulfonate, fumarate could act as an apparent terminal electron acceptor in propionate oxidation. ^{13}C-nuclear magnetic resonance experiments showed that propionate was carboxylated to succinate while fumarate was partly oxidized to acetate and partly reduced to succinate. Fumarate alone was fermented to succinate and CO_2. Bacteria growing on fumarate were enriched and obtained free of methanogens. Propionate was metabolized by these bacteria when either fumarate or *Methanospirillum hungatii* was added. In cocultures with *Syntrophobacter wolinii*, such effects were not observed upon addition of fumarate. Possible slow growth of *S. wolinii* on fumarate could not be demonstrated because of the presence of a *Desulfovibrio* strain which grew rapidly on fumarate in both the absence and presence of sulfate.

Propionate is an important intermediate in the conversion of complex organic carbon to methane and carbon dioxide. Up to 15% of total methanogenesis is derived from the degradation of propionate to acetate and carbon dioxide (10, 19). Propionate oxidation is accomplished by obligate syntrophic consortia of proton-reducing acetogenic bacteria and methanogenic bacteria (1, 8, 10, 19). Because propionate oxidation to acetate and carbon dioxide is energetically very unfavorable, methanogens or sulfate-reducing bacteria are needed to make propionate oxidation feasible either by hydrogen consumption (1, 4, 9, 10) or by formate consumption (3, 25). A few syntrophic propionate-oxidizing cultures have been described (2, 4, 17, 19–21). Thus far, *Syntrophobacter wolinii* is the only described propionate-oxidizing bacterium which was obtained in a defined coculture with a *Desulfovibrio* strain (2). Syntrophic propionate-oxidizing bacteria are highly specialized; propionate is thought to be the only substrate for this type of organism. Because propionate-oxidizing bacteria cannot be grown on propionate in the absence of hydrogenotrophs, their biochemical and physiological properties are difficult to assess.

Labelling experiments with ^{13}C- and ^{14}C-labelled substrates and enzyme measurements in cell extracts of syntrophic cultures have shown that the methylmalonyl-coenzyme A (CoA) pathway is involved in propionate oxidation by syntrophic cultures (13–15, 17, 19, 21). In this route, propionyl-CoA, methylmalonyl-CoA, succinyl-CoA, succinate, fumarate, malate, oxaloacetate, pyruvate, and acetyl-CoA are intermediates. The carboxylation of propionyl-CoA is coupled to the decarboxylation of oxaloacetate by means of a transcarboxylase (14, 15). When the different steps of syntrophic propionate oxidation are compared (Table 1), it is obvious that oxidation of succinate to fumarate coupled to hydrogen formation is the most difficult step in propionate oxidation. It might be advantageous for syntrophic propionate-oxidizing bacteria to omit succinate oxidation when fumarate is present. Propionate would then be converted to succinate, while fumarate is oxidized to acetate. Research was started to investigate the effect of fumarate on propionate oxidation by syntrophic cultures. Results with a newly enriched propionate-oxidizing methanogenic culture and with sulfidogenic and methanogenic cultures with *S. wolinii* are presented.

MATERIALS AND METHODS

Organisms. A newly enriched propionate-oxidizing methanogenic culture originating from the work of Jan Dolfing at our department (7) is described herein. The *S. wolinii-Desulfovibrio* strain G11 coculture (DSM 2805) and *Methanospirillum hungatii* JF-1 (DSM 864) were obtained from the Deutsche Sammlung von Mikroorganismen (Braunschweig, Germany). An *S. wolinii-M. hungatii* culture, in which *Desulfovibrio* strain G11 was present only as a contaminant, was obtained by subculturing in propionate-media without sulfate.

Media and cultivation. A basal bicarbonate-buffered medium contained the following (grams per liter): $Na_2HPO_4 \cdot 2H_2O$, 0.53; KH_2PO_4, 0.41; NH_4Cl, 0.3; $CaCl_2 \cdot 2H_2O$, 0.11; $MgCl_2 \cdot 6H_2O$, 0.10; NaCl, 0.3; $NaHCO_3$, 4.0; and $Na_2S \cdot 9H_2O$, 0.48 (as well as acid and alkaline trace elements [each, 1 ml/liter] and vitamins [0.2 ml/liter]). The acid trace element solution contained the following (millimolar): $FeCl_2$, 7.5; H_3BO_4, 1; $ZnCl_2$, 0.5; $CuCl_2$, 0.1; $MnCl_2$, 0.5; $CoCl_2$, 0.5; $NiCl_2$ 0.1; and HCl, 50. The alkaline trace element solution was composed of the following (millimolar): Na_2SeO_3, 0.1; Na_2WO_4, 0.1; Na_2MoO_4, 0.1; and NaOH, 10. The vitamin solution had the following composition (gram per liter): biotin, 0.02; niacin, 0.2; pyridoxine, 0.5; riboflavin, 0.1; thiamine, 0.2; cyanocobalamin, 0.1; *p*-aminobenzoic acid, 0.1; and pantothenic acid, 0.1. Media for the coculture with *S. wolinii* were supplemented with 0.02% BBL yeast extract (Becton Dickinson, Cockeysville, Md.) and 2.8 g of Na_2SO_4 per liter, and media for *M. hungatii* were supplemented with 0.02% BBL

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TABLE 1. Reaction steps involved in propionate oxidation via the methylmalonyl-CoA pathway[a]

Propionate oxidation via the methylmalonyl-CoA pathway	$\Delta G^{\circ\prime}$	$\Delta G^{\prime}$[b]
Overall reaction, $propionate^- + 3H_2O \rightarrow acetate^- + HCO_3^- + 3H_2$	+76.1	−10.9
Reaction step		
$Propionate^- + HCO_3^- \rightarrow succinate^{2-} + H_2O$	+20.5	+20.5
$Succinate^{2-} \rightarrow fumarate^{2-} + H_2$	+86.2	+57.2
$Fumarate^{2-} + H_2O \rightarrow malate^{2-}$	−3.7	−3.7
$Malate^{2-} \rightarrow oxaloacetate^{2-} + H_2$	+47.7	+18.7
$Oxaloacetate^{2-} + H_2O \rightarrow pyruvate^- + HCO_3^- + H^+$	−27.2	−27.2
$Pyruvate^- + 2H_2O \rightarrow acetate^- + HCO_3^- + H^+ + H_2$	−47.3	−76.3

[a] Standard Gibbs free energy changes were obtained or calculated from the data of Thauer et al. (24).
[b] Partial H_2 pressure = 10^{-5} atm (1 atm = 101.29 kPa).

yeast extract, 0.04% bioTrypticase (bioMérieux, Charbonnieres les Bains, France), and 0.15 g of sodium acetate per liter. Routinely, bacteria were cultured at 37°C in 120-ml serum vials with 50 ml of medium and a gas phase of 1.7 atm (172.2 kPa) N_2-CO_2 or H_2-CO_2 (80:20, vol/vol). Bottles were sealed with butyl rubber stoppers (Rubber BV, Hilversum, The Netherlands) and aluminum caps. Vitamins and bromoethanesulfonate (BrES) were filter sterilized; other compounds were sterilized by heat. Unless stated otherwise, substrates were added from 1 M stock solutions to give a final concentration of 20 mM. In most experiments, media were inoculated with stock cultures which had been stored between 15 and 20°C. Growth rates were estimated by monitoring the optical density at 600 nm or product formation of duplicate bottles. For this, substrate-adapted and actively growing cultures were inoculated (the inoculum size was 10%) in fresh media. Experiments to determine CO_2 formation were done with phosphate-buffered media. Bicarbonate (50 mM) was replaced by 30 mM sodium phosphate (pH 7), and the gas phase was N_2 instead of N_2-CO_2.

Analytical methods. Organic acids were measured with an LKB high-performance liquid chromatograph (HPLC) equipped with a Chrompack organic acid column (30 cm by 6.5 mm). The mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.6 ml/min. The column temperature was 60°C. Samples (20 μl) were injected by using a Spectra Physics autosampler (SP 8775). Compounds eluting were quantified by differential refractometry by an LKB 2142 refractometer. Acetate, propionate, and other fatty acids were also measured by gas chromatography (GC) with a CP9000 gas chromatograph (Chrompack, Middelburg, The Netherlands) equipped with a glass column (inside dimensions, 180 cm by 2 mm) filled with Chromosorb 101 (80/100 mesh). The carrier gas was nitrogen saturated with formic acid. The temperatures of the injection port and the detector were 250 and 300°C, respectively. The temperature of the column was maintained at 160 or 180°C. With both HPLC and GC the detection limit for compounds was about 0.1 mM. Methane and hydrogen levels were measured by GC with a Packard-Becker 417 gas chromatograph equipped with a thermal conductivity detector and molecular sieve 13× (60/80 mesh). The column temperature was 50°C, and the carrier gas was argon at a flow rate of 20 ml/min. CO_2 levels were measured by GC in a fashion similar to that used for the other gases. However, a Poropak Q column was used, and helium was the carrier gas. Bicarbonate was determined by the same method as CO_2; culture samples (2.5 ml) were injected into closed 18-ml Hungate tubes, and 2.5 ml of 1 M HCl was added to purge the CO_2 from the liquid phase. L-Malate was measured enzymatically with L-malate dehydrogenase (11).

Nuclear magnetic resonance experiments. Nuclear magnetic resonance experiments were performed as follows. Media (5 ml) were prepared in 18-ml Hungate tubes. In experiments to demonstrate involvement of the methylmalonyl-CoA pathway, media contained 20 mM [3-^{13}C]propionate or 10 mM [2,3-^{13}C]propionate plus 10 mM unlabeled propionate. In experiments to study the fate of propionate in the presence of fumarate, media contained 20 mM [3-^{13}C]propionate and 10 mM BrES in the presence or absence of 40 mM fumarate. [3-^{13}C]propionate and [2,3-^{13}C]propionate (minimum of 99 atom% ^{13}C) were obtained from Isotec (Miamisburg, Md.). Tubes were inoculated (10%) with the propionate-oxidizing culture and incubated for 2 weeks at 37°C. Cells were centrifuged, and D_2O (10%, vol/vol) was added to the supernatant. ^{13}C-labelled compounds were analyzed with a Bruker CXP-300 Fourier-Transform spectrometer as described previously (13).

Other methods. Phase-contrast photographs were made with a Wild phase-contrast microscope. Gram staining was done according to standard procedures (6).

RESULTS

Origin and description of the newly enriched culture. The propionate-oxidizing culture originated from the research of Dolfing (7). Methanogenic granular sludge of a bench-scale upflow anaerobic sludge blanket reactor, which had been operated for several months with a mixture of propionate and acetate as feed, was used as starting material for the enrichment of propionate-oxidizing bacteria. The culture was enriched in 1983 and regularly subcultured in media with propionate as the sole substrate. Thereafter, it had been stored for about 2 years at room temperature in the dark before subculturing was continued in 1987. The propionate-degrading culture mainly consisted of gram-negative, short, rod-shaped bacteria (1.1 to 1.6 by 1.8 to 2.5 μm) and a *Methanospirillum* sp. (about 0.8 μm in width). The culture was highly purified but was not yet a defined coculture. Even after repeated transfer and dilution in fresh media, small coccoid bacteria remained present in low numbers (less than 1%). These contaminants could be enriched and isolated with yeast extract as a substrate. Yeast extract was fermented by this organism to acetate, isobutyrate, butyrate, and isovalerate (or β-methylbutyrate) in a molar ratio of about 20:2:3:6. This bacterium was also able to grow on glucose or pyruvate. However, it was unable to grow on propionate, succinate, malate, or fumarate, neither in the absence nor in the presence of *M. hungatii* JF-1 (DSM 864). It is possible that the coccoid bacterium is maintained in the propionate-oxidizing culture by growth on cell lysis prod-

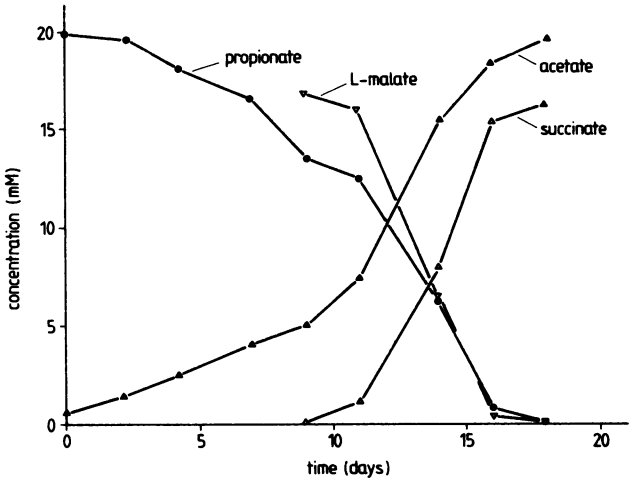

FIG. 1. Effect of L-malate on propionate oxidation by the newly enriched syntrophic culture. L-Malate was added to the culture at day 9.

ucts. The *Methanospirillum* sp. could be enriched with hydrogen plus carbon dioxide or formate as energy substrates. This methanogen morphologically and physiologically resembled *M. hungatii* JF-1 (DSM 864). In our media, both strains had an absolute requirement for bioTrypticase, and growth was stimulated by addition of sodium acetate (2 mM) to the media. Remarkably, the *Methanospirillum* sp. did not require bioTrypticase during syntrophic growth with propionate-oxidizing bacteria, which suggests the occurrence of cross-feeding. Bacteria with other morphologies were never observed. The propionate-oxidizing culture converted propionate in the expected stoichiometry: 24.1 ± 1.0 mol of propionate yielded 23 ± 1.8 mol of acetate and 17.3 ± 0.7 mol of methane. The apparent maximum specific growth rate, estimated from the increase in acetate concentration in time, was between 0.15 and 0.17 day^{-1}. Labelling experiments showed that [3-^{13}C]propionate was converted to equal amounts of [1-^{13}C]- and [2-^{13}C]acetate. In addition, [2,3-^{13}C]propionate in the presence of unlabelled propionate was solely converted to [1,2-^{13}C]acetate (results not shown).

Effect of fumarate and malate on propionate oxidation. Addition of L-malate to the propionate-oxidizing culture led to an instantaneous and stoichiometric formation of succinate (Fig. 1). When DL-malate was added, only the L-isomer was consumed. Succinate formation was also observed with fumarate, whereas D-malate, malonate, and maleate were not metabolized. All of the observations presented below were obtained with fumarate, but similar results were also obtained with L-malate. These two compounds were interconverted to some extent in the culture. Table 2 shows that in presence of fumarate less methane was produced per amount of propionate which had been oxidized. Addition of BrES, a specific inhibitor of methanogenesis, led to a complete inhibition of propionate oxidation. However, in BrES-inhibited cultures, propionate was converted when fumarate was present as well. These observations suggested that hydrogen consumption by methanogens can be replaced by addition of fumarate. Dense hydrogen-pregrown cultures of *M. hungatii* JF-1 (DSM 864) and the *Methanospirillum* sp. were unable to reduce fumarate to succinate with hydrogen or formate as the electron donor in the presence of BrES. This shows that the methanogens do not shift their metabolism from methane formation to fumarate reduction when methanogenesis is inhibited in the propionate-oxidizing culture. In BrES-inhibited propionate-oxidizing cultures [3-^{13}C]propionate is randomized to some extent to [2-^{13}C]propionate, while no net degradation of propionate occurred (Fig. 2B). However, when fumarate is present in BrES-inhibited cultures, label originating from propionate was mainly recovered in succinate, while no label in acetate above the natural occurring background level of 1.1% was recovered (Fig. 2A). This shows that the acetate must have been formed from the unlabeled fumarate.

TABLE 2. Effect of fumarate on propionate oxidation by a mesophilic enrichment culture[a]

Culture[b]	Substrate conversion and product formation (mmol/liter)				
	Propionate degraded	Acetate formed	Methane formed	Fumarate degraded	Succinate formed
Propionate	18.4	19.2	12.3		
Propionate + BrES	0.0	0.7	0.0		
Propionate + fumarate	17.6	17.6	4.8	37.3	36.9
Propionate + fumarate + BrES	12.0	12.4	0.0	35.4	34.6
Fumarate				36.0	29.9

[a] Similar results were obtained when L-malate instead of fumarate was used.

[b] The initial concentrations of propionate and fumarate were about 18 and 36 mM, respectively; the BrES concentration was 10 mM. Cultures were incubated for 4 weeks at 37°C.

Growth on fumarate as sole substrate. Fumarate in the

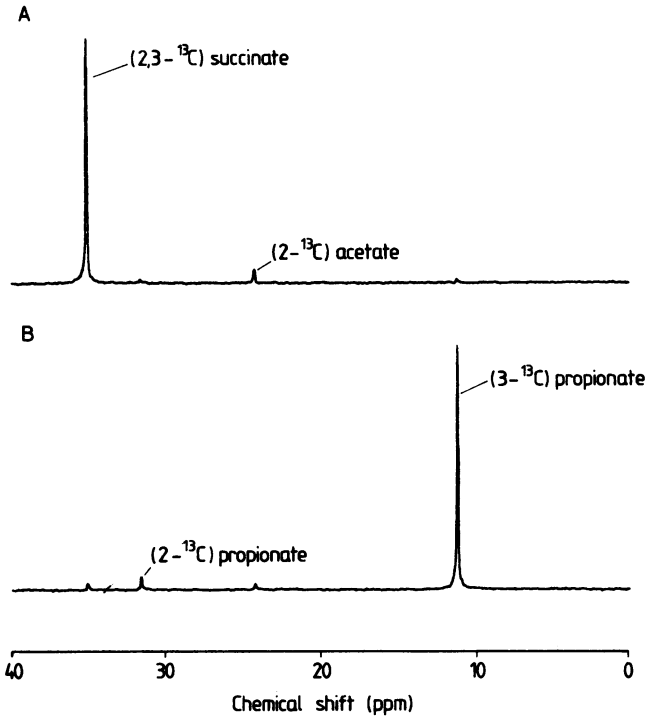

FIG. 2. Nuclear magnetic resonance spectra of culture supernatants of the newly enriched propionate-oxidizing culture incubated with 20 mM [3-^{13}C]propionate, 40 mM fumarate, and 10 mM BrES (A) or incubated with 20 mM [3-^{13}C]propionate and 10 mM BrES (B).

absence of propionate was degraded as well (Table 2). Fumarate degradation was coupled to growth, and compared with syntrophic growth on propionate, high cell densities were obtained. Succinate was the sole organic end product detected by HPLC and GC methods (the detection limit of compounds was about 0.1 mM). Because reducing equivalents are needed for conversion of fumarate to succinate, part of the fumarate must have been oxidized to carbon dioxide. Formation of carbon dioxide was confirmed by using phosphate-buffered media. In this case, we found that 25.2 mmol of fumarate was converted into 19.5 mmol of succinate and 15.5 mmol of CO_2. Similar values were found in the presence of BrES, and only minor amounts of methane were formed in the absence of BrES. By repeated transfer and dilution in fumarate media, a microscopically homogeneous culture was obtained. Methanogens were completely removed; even after months of incubation, methane formation from hydrogen or formate did not occur. The bacteria growing on fumarate resembled the propionate-oxidizing bacteria in the propionate-degrading culture. However, the average size was somewhat larger (1.3 to 1.8 by 1.8 to 3.8 μm). The coccoid contaminant remained present in the culture in very low numbers. The fumarate-degrading culture grew best when dense inocula of 5 to 10% were used. When a small inoculum (1% or less) was used, cultures had a long lag phase before growth started. Later experiments showed that the lag phase could be shortened considerably by adding 0.1 mM dithionite to the media, suggesting that a low redox potential is beneficial for growth. Growth on fumarate was relatively slow; the estimated maximum specific growth rate was about 0.20 day^{-1}. The fumarate-degrading culture fermented fumarate plus propionate to succinate plus acetate in the same fashion as described above for the BrES-inhibited propionate-oxidizing culture, and in presence of hydrogen or formate, fumarate was stoichiometrically transformed to succinate. Under these conditions, growth was slower than with fumarate alone. The estimated specific growth rate in media with propionate plus fumarate (20 and 60 mM, respectively) was about 0.16 day^{-1}. The culture, which was enriched with fumarate, was unable to degrade propionate in the absence of fumarate. However, propionate was degraded when *M. hungatii* JF-1 (DSM 864) was added (Fig. 3). In these experiments, it was confirmed that the methanogen alone was not able to degrade propionate. We tried to isolate fumarate-degrading bacteria with fumarate (20 mM) or with fumarate (40 mM) plus propionate (20 mM) in agar media in roll tubes or in agar shake cultures. These attempts were not successful, because the bacteria failed to form colonies. Addition of yeast extract (0.1%) to the media led to colonies of the contaminant only.

Fumarate utilization by cultures with *S. wolinii*. The effect of fumarate on propionate oxidation by a methanogenic and a sulfidogenic culture with *S. wolinii* was investigated. In both cultures, *Desulfovibrio* strain G11 was present either as the hydrogenotroph (sulfidogenic culture) or as a contaminant (methanogenic culture). Like in the original description of *S. wolinii*, we were unable to get a defined coculture of the proton-reducing acetogen with *M. hungatii* (2). The *Desulfovibrio* strain was able to degrade fumarate both in the presence and in the absence of sulfate. In the absence of sulfate, strain G11 fermented fumarate to succinate and acetate in the expected stoichiometries, whereas in the presence of sulfate (results not shown) or *M. hungatii*, less succinate and more acetate were formed (Table 3). The *Desulfovibrio* sp. grew only very slowly in media with succinate and sulfate, whereas we did not observe syntrophic growth of the *Desulfovibrio* sp. on succinate in the absence of sulfate but the presence of *M. hungatii* (results not shown). The sulfidogenic coculture was unable to degrade propionate in the presence of fumarate in sulfate-free media. However, fumarate was fermented rapidly to acetate and succinate. This suggested that, under those conditions, interspecies electron transfer does not occur. Thus, the sulfate reducer preferred fermentative growth on fumarate above growth by fumarate reduction with reducing equivalents derived from propionate oxidation by *S. wolinii*. In the methanogenic propionate-oxidizing culture, the *Desulfovibrio* sp. was present only in low cell numbers (about 1%). Propionate, fumarate, and fumarate plus propionate were mainly converted to acetate by this methanogenic propionate-oxidizing culture, and methane was produced in the expected amounts (results not shown). This indicates that the two substrates were both degraded to acetate via interspecies electron transfer. The *Desulfovibrio* sp. was enriched in the media with fumarate as the substrate, whereas the numbers of *S. wolinii* increased with propionate as the substrate. When propionate and fumarate were both present, increased numbers of both species were found. Because of the fast growth of the *Desulfovibrio* sp. on fumarate, a possible slow conversion of fumarate by *S. wolinii* could not be assessed.

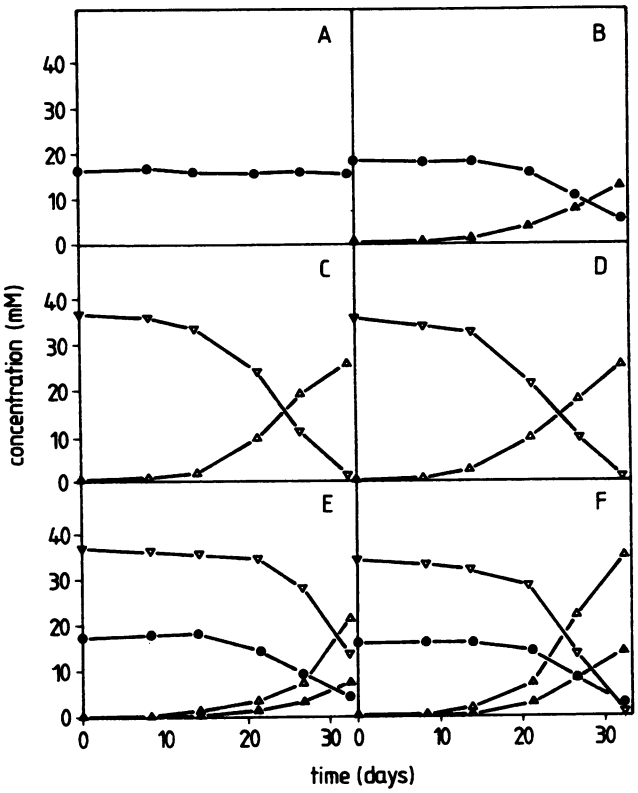

FIG. 3. Propionate (●) and fumarate (▽) degradation and acetate (▲) and succinate (△) formation by bacteria enriched with fumarate as substrate. Cultures were made in the absence (A, C, and E) or presence (B, D, and F) of *M. hungatii*.

DISCUSSION

Three important observations were made when fumarate (or malate) was added to a newly enriched syntrophic

TABLE 3. Fumarate and propionate utilization by mixed cultures of *S. wolinii*, *Desulfovibrio* strain G11, and *M. hungatii* JF-1 in media without sulfate

Culture[a]	Substrate conversion and product formation (mmol/liter)			
	Propionate degraded	Acetate formed	Fumarate degraded	Succinate formed
Syntrophobacter + *Desulfovibrio*				
Fumarate		7.2	18.4	10.5
Propionate	1.0	0.7		
Propionate + fumarate	2.3	9.0	17.6	10.6
Syntrophobacter + *Methanospirillum*[b]				
Fumarate		15.8	18.0	1.7
Propionate	18.8	18.9		
Propionate + fumarate	18.4	31.0	16.9	2.2
Desulfovibrio, fumarate		6.8	16.5	10.5
Desulfovibrio + *Methanospirillum*, fumarate		12.4	16.7	4.3

[a] The initial concentrations of propionate and fumarate were about 18 mM each. The cultures were incubated for 4 weeks at 37°C.
[b] In this culture, the *Desulfovibrio* sp. was still present as a contaminant.

propionate-oxidizing culture. (i) The difficult succinate-oxidation step is avoided. (ii) Fumarate could replace methanogens as an apparent electron acceptor in propionate oxidation. (iii) Fumarate in the absence of propionate was fermented to succinate and carbon dioxide. From the experiments with labelled propionate, it is evident that in media with fumarate plus propionate, the following sequence of reactions takes place in the propionate-oxidizing methanogenic culture: [3-^{13}C]propionate + CO_2 → [2-^{13}C]succinate, 1 fumarate → 1 acetate + $2CO_2$ + 4[H], and 2 fumarate plus 4[H] → 2 succinate. Thus, propionate is not oxidized to acetate but is solely carboxylated to succinate. Carboxylation of propionate to succinate is an energy-requiring reaction; the $\Delta G^{\circ\prime}$ is +20.5 kJ/mol. Therefore, this conversion is possible only if it is coupled to another energy-yielding reaction. A similar carboxylation of propionate to succinate was recently demonstrated for *Propionibacterium freudenreichii* (22). This propionigenic bacterium contains a propionyl-CoA–oxaloacetate transcarboxylase, an enzyme which drives the endergonic carboxylation of propionyl-CoA to methylmalonyl-CoA by the exergonic decarboxylation of oxaloacetate to pyruvate. This bacterium lacks an oxaloacetate decarboxylase, and therefore it can ferment fumarate only to succinate and acetate when propionate is present as a carboxyl acceptor.

Growth of the propionate-oxidizing culture on fumarate alone was rather unexpected. We made use of this property to obtain a culture which was microscopically pure and completely free of methanogens. This highly purified culture fermented fumarate to succinate and CO_2 according to the sequence 7 fumarate → 6 succinate + $4CO_2$, as was also found for some other fumarate-fermenting anaerobes (5, 18). In the presence of hydrogen or formate fumarate was stoichiometrically converted to succinate. The culture carboxylated propionate to succinate, while fumarate was no longer oxidized to CO_2 but to acetate. In addition, the culture oxidized propionate to acetate in the presence of *M. hungatii* JF-1 (DSM 864), an organism which is unable to metabolize propionate. The fact that only one morphological type of bacterium was present under either growth condition may indicate that the syntrophic propionate-oxidizing bacteria are involved in all of these reactions. Besides, the shift from CO_2 to acetate formation from fumarate in the presence of propionate and the simultaneous energy-dependent carboxylation of propionate can be explained satisfactorily only if one bacterial species is involved. However, direct evidence for this is possible only after a pure culture has been obtained. Thus far, we did not succeed because the propionate-oxidizing bacteria failed to grow in solid media, and with serial dilutions in liquid media we were not able to get rid of a contaminating fermenting organism with characteristic morphology. We were able to isolate this contaminant and could show that its metabolism does not interfere with the fumarate and propionate metabolism of the propionate-oxidizing bacteria.

Thus far, proton-reducing propionate-oxidizing bacteria were thought to use only propionate for growth. Because this substrate is degraded in syntrophy with hydrogenotrophic bacteria, detailed biochemical and physiological studies with these proton-reducing acetogens are not possible, the more so because attempts to grow syntrophic propionate-oxidizing bacteria in the absence of methanogens with artificial electron removal systems were not successful (16). Our findings could make syntrophic propionate-oxidizing bacteria accessible for further study.

It remains to be studied in detail whether fumarate utilization by propionate-oxidizing bacteria is restricted to a few species of syntrophic propionate-oxidizing bacteria. Our earlier findings (12) with a propionate-oxidizing culture enriched by Koch et al. (17) and results presented here with *S. wolinii* suggest that fumarate utilization is not common among syntrophic propionate-oxidizing bacteria. However, in a study with a highly purified thermophilic syntrophic culture, we have obtained evidence for an advantageous effect of fumarate on propionate oxidation. In the presence of fumarate, this thermophilic culture carboxylated propionate to succinate and oxidized fumarate to acetate in a fashion similar to the culture described here, but growth with fumarate alone or propionate oxidation coupled to fumarate reduction was not found (23).

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