APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1985, p. 1244–1250
0099-2240/85/111244-07$02.00/0

Vol. 50, No. 5

# Interactions in Syntrophic Associations of Endospore-Forming, Butyrate-Degrading Bacteria and $H_2$-Consuming Bacteria

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Received 13 May 1985/Accepted 23 August 1985

**Butyrate is an important intermediate in the anaerobic degradation of organic matter. In sulfate-depleted environments butyrate is oxidized to acetate and hydrogen by obligate proton reducers, in syntrophic association with hydrogen-consuming methanogens. This paper describes two enrichments of endospore-forming bacteria degrading butyrate in consortia with methanogens. The isolates are readily established in coculture with $H_2$-consuming, sulfate-reducing bacteria by pasteurizing the culture. The two original enrichments differed in that one grew to an optically dense culture while the second grew in clumps. Examination by scanning electron microscopy showed that clumping resulted from the production of large amounts of extracellular polymer. Several $H_2$-consuming methanogens were identified in the enrichments. Some of them grew closely associated to the butyrate degraders. This attachment to the hydrogen producer may permit some methanogens to compete for the growth substrate against other bacteria having higher substrate affinity.**

Volatile fatty acids are important intermediates in environments where methanogenesis is the main terminal dissimilatory process. For example, compounds such as propionate and butyrate account for nearly 15 and 5%, respectively, of the methane that is produced in anaerobic digesters (15, 21). These compounds are not dissimilated by either fermentors or methanogens, but by a third group of bacteria, known as obligate proton reducers (8, 43). These bacteria must regenerate their coenzymes by the reduction of protons to molecular hydrogen, a mechanism that is thermodynamically unfavorable under standard conditions.

$$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$$
$$\Delta G^{0\prime} = +48.1 \text{ kJ}$$

$$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$$
$$\Delta G^{0\prime} = +76.1 \text{ kJ}$$

The free energy of these reactions is positive unless the end product concentrations are maintained (by removal) below certain critical levels (16).

Several obligate proton reducers have been described. The S organism was isolated from "*Methanobacillus omelianskii*" a consortium of two bacteria, one degrading ethanol to acetate and $H_2$:

$$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + H_2$$
$$\Delta G^{0\prime} = +9.6 \text{ kJ}$$

and the second (a methanogen) growing on the $H_2$ produced by the first bacterium (9). The second and third bacteria described were *Syntrophomonas wolfei* and *Syntrophobacter wolinii*. *Syntrophomonas wolfei* oxidizes even-numbered fatty acids to acetate and $H_2$ and oxidizes odd-numbered fatty acids to propionate, acetate, and $H_2$ (23, 24). *Syntrophobacter wolinii* oxidizes propionate to acetate, $CO_2$, and $H_2$ (7). Recently, an obligate proton reducer capable of degrading benzoate in consortia with methanogens was described (31, 32).

Maintenance of a low partial pressure of hydrogen is critical for growth. The S organism grew poorly in pure culture on ethanol. The fatty acid degraders, not yet isolated in pure culture, must be grown in coculture with a $H_2$-oxidizing bacterium, e.g., a sulfate reducer or a methanogen. Although the thermodynamics of the reaction are determined by the relative concentrations of substrates and products, the removal of $H_2$ is the critical step. To date, no obligate proton reducers have been grown by removing acetate alone. All known isolates have been obtained by removing the $H_2$.

We describe here two enrichment cultures of spore-forming bacteria that degrade butyrate in syntrophic association with methanogens. Shelton and Tiedje (39) and Stieb and Schink (41) have also recently isolated spore formers capable of carrying out the same process. We show that the spore formers can be readily isolated and established in coculture with $H_2$-oxidizing bacteria by pasteurizing the cultures. The pasteurization process provides the tool for the isolation of the obligate proton reducers in pure culture, if alternate means to remove the $H_2$ are found. Some of the methanogens associated with the spore formers grew as individual cells, while others grew in clumps attached to the butyrate degrader. It is hypothesized that attachment is an important strategy in interspecific $H_2$ transfer.

## MATERIALS AND METHODS

**Minimal medium.** A sulfate-free medium (44) was used throughout this study as the basis for all growth media. Inorganic salts were added from refrigerated stock solutions. Energy sources, electron acceptors, and other nutrient additions were added directly from the shelf. The reducing agents, sulfide and dithionite, were freshly prepared by dissolution in distilled water previously degassed with $N_2$.

The media were prepared by mixing all the components, except the reducing agents, in a 1-liter stoppered serum bottle and degassing at least three times with 80% $N_2$–20% $CO_2$. The medium was then reduced and dispensed by sterile

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filtration with Millex-GV filter units (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.) into 50-ml serum bottles. The latter had been previously degassed with 80% $N_2$–20% $CO_2$ or 80% $H_2$–20% $CO_2$ and sterilized in the autoclave. The medium equilibrated to a pH of 6.6 to 6.7 without the need of further acid-base additions.

The 1-liter serum bottles were prepared by the method of Balch and Wolfe (4). The 50-ml serum bottles and the serum tubes have been described by Miller and Wolin (26). Transfer of the medium to the growth vessels was done as performed by Baresi and Wolfe (5), except that a membrane filter was added to the setup. All gases were passed over hot copper kept at 320°C to remove traces of oxygen (14). All gas exchanges were done in a system described by Balch and Wolfe (3).

**Bacterial strains.** *Desulfovibrio vulgaris* Marburg (DSM 2119) was obtained from the German Collection of Microorganisms. *Desulfovibrio vulgaris* Madison was obtained from the bacterial collection of the Department of Bacteriology, University of Wisconsin, Madison. The two strains grow optimally at 35 to 37°C (1). *Desulfovibrio* sp. strain CGSO4 was isolated from an anaerobic filter processing coal gasification waste (10). Isolate RotseeSO4, not assigned yet to a genus, was isolated from the anaerobic hypolimnion of a Swiss lake (18). Strains CGSO4 and RotseeSO4 grow optimally at 40 and 30°C, respectively. Cells were regularly maintained in the defined salts mineral medium, supplemented with 10 mM $SO_4^{2-}$ and 1 mM sodium acetate, under an atmosphere of 80% $H_2$–20% $CO_2$.

**Volatile fatty acids.** Volatile fatty acids were determined as outlined by Holdeman et al. (13). Samples (0.5 ml) were withdrawn with a tuberculin syringe and placed in 2-ml vials equipped with teflon-lined caps (Wheaton Scientific, Millville, N.J.). The acids were extracted with ethyl ether after adding NaCl and acidifying with $H_2SO_4$. After extraction, the water layer was withdrawn with a syringe, and the ether extract was dried over anhydrous $CaCl_2$.

Samples were injected with a 10-μl syringe into a Hewlett-Packard 5711 gas chromatograph fitted with a flame ionization detector. A stainless steel column (length, 1.8 m; outer diameter, 3 mm) packed with 10% SP-1000–1% $H_3PO_4$ on 100/120 Chromosorb W AW (Supelco Inc., Bellefonte, Pa.) was used. An oven temperature of 110°C was maintained for 2 min and then programmed from 110 to 170°C at 32°C $min^{-1}$. The injector temperature was 150°C, and the detector temperature was 200°C. The carrier gas flow was maintained at 30 ml of $N_2$ $min^{-1}$. The concentration of each compound was determined by comparing the peak height with that of a standard of volatile acids (Supelco Inc.).

**Scanning electron microscopy.** Cells were fixed overnight with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and collected on a filter (pore size, 0.2 μm; Nuclepore Corp., Pleasanton, Calif.). After the excess fixative was washed off with distilled water, the filters were folded, placed inside plastic canisters, and dehydrated in an ethanol or acetone gradient. The dehydrated filters were critical point dried with $CO_2$, unfolded and taped to metal stubs. Coating was done with Au-Pd. The scanning electron microscopy observations were made with an AMR 1000 microscope.

**Transmission electron microscopy.** Bacteria were prepared for transmission electron microscopy by the method of Zeikus and Bowen (45). Cells were washed in 0.074 M cacodylate buffer (pH 7.3) and centrifuged. The cell pellet was suspended and fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3) for 10 h at room temperature. The cells were washed in the same buffer, pelleted, and suspended in 1.5% Noble agar at 50°C. The agar-cell suspension was cooled and cut into small cubes. The cubes were fixed for 7 h in 1% $OsO_4$ in 0.07 M cacodylate buffer and then washed twice in the same buffer. The fixed agar cubes were dehydrated through a graded ethanol series and placed in propylene oxide. Cubes were embedded in a low-viscosity resin (40). The blocks were thin sectioned with a diamond knife and an ultramicrotome. Thin sections were stained with uranyl acetate and lead citrate (37) and examined in a Zeiss transmission electron microscope. The same fixation procedure was also performed with 0.05% ruthenium red added at each step after the first wash, up to midway through the dehydration.

## RESULTS

**Isolation of a butyrate-degrading consortium from coal gasification waste.** Liquor from a pilot-scale, up-flow anaerobic filter processing waste from a coal gasification plant (10) was inoculated (10% [vol/vol]) into the basic salts mineral medium supplemented with 25 mM sodium butyrate and 10 mM sodium sulfate. The enrichment was incubated at 37°C until a dense culture was obtained and then transferred to a sulfate-free medium containing butyrate as the sole energy source. The medium was not supplemented with other organic carbon sources except for vitamins. The use of such a medium reduces the growth of fermentative bacteria. No further efforts were made to isolate butyrate-degrading, sulfate-reducing bacteria.

Microscopic observations of the sulfate-free enrichment showed that it was composed primarily of fluorescent bacteria resembling *Methanospirillum hungatei* and *Methanosarcina barkeri*. Straight, nonfluorescent rods with truncated ends similar to *Methanothrix soehngenii* and straight fluorescent rods similar to *Methanobacterium* spp. were also observed.

Continued transfer by serial dilution resulted in the dominance of the culture by three types of bacteria. An unidentified slightly curved, spore-forming rod and *Methanospirillum* sp. were the first to grow (Fig. 1A). A sarcina appeared in large numbers after most of the butyrate had been consumed, suggesting that in the enrichment, its main growth substrate was acetate rather than $H_2$. A *Methanobacterium* sp. was observed in lower numbers than *Methanospirillum*, usually attached to small clumps of cells.

Incubation of the enrichment cultures at different temperatures showed that the optimum temperature of the consortium was 40°C (Fig. 2A). Butyrate and acetate were the only two volatile acids detected in the medium. After 25 days, no sign of growth was observed after incubations at 45 or 50°C or below 30°C. After 2 months, small clumps of bacteria could be detected in the medium that had been incubated at 45°C. Microscopic observation showed that the spore former and the straight fluorescent rod, observed in lower numbers below 40°C, grew attached to each other in the clumps in an otherwise clear culture. The fluorescent rod continued to grow in clumps when transferred to $H_2$-$CO_2$ or formate medium. This bacterium appeared to be a strain of *Methanobacterium formicicum*, because among the long, mesophilic rods only *Methanobacterium formicicum* strains grow on formate and $H_2$ (2).

Because the probable butyrate-degrading bacterium was a spore former, we were able to pasteurize the medium at 80°C for 15 min without killing the organism. After serial dilution of the pasteurized medium and reinoculation with pure cultures of $H_2$-oxidizing, sulfate-reducing bacteria, the same

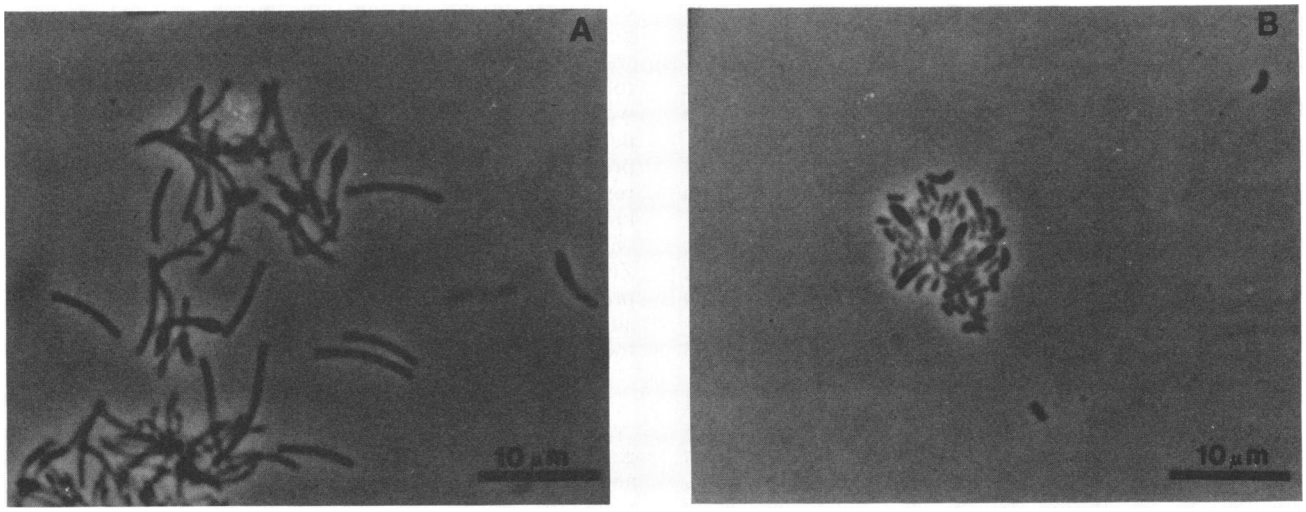


FIG. 1. (A) Phase-contrast micrograph from butyrate enrichment of coal gasification waste. *Methanospirillum* sp. and the butyrate degrader, the latter showing early development of spores, are visible. (B) Phase-contrast micrograph from butyrate enrichment from the Charles River. Translucent structures are spores. The short, straight rod is a methanogen and the curved rod in the periphery is *Desulfovibrio vulgaris* Madison.

morphologically similar spore former grew regardless of the sulfate-reducing strain added to the coculture. Best growth was obtained when the pasteurized culture was coinoculated with *Desulfovibrio* sp. strain (CGSO4, isolated from the same enrichment culture, into butyrate-sulfate medium (Fig. 3A). Optically dense cultures of this consortium were observed after several days in the $10^{-6}$ dilution but not in the $10^{-7}$ dilution.

When a sulfate reducer isolated from an anaerobic Swiss lake was inoculated (Fig. 3B, isolate RotseeSO4), the cells grew in discrete clumps in an otherwise clear culture. Inoculation of *Desulfovibrio vulgaris* Madison or Marburg produced a combination of the two modes of growth. No growth occurred initially when *Desulfovibrio vulgaris* was cocultured with a $H_2$-oxidizing, sulfate-reducing bacterium isolated from tropical marine sediments, but eventually it also grew with this isolate. It is interesting to note that isolate RotseeSO4 grew in small clumps and tended to attach reversibly to the glass surface of the growth vessel. The marine isolate grew in tightly packed clumps and produced large amounts of extracellular polymer. When these two isolates were grown with the butyrate degrader, the same types of clumps formed with the butyrate degrader trapped within them.

Transfer of the cocultures of the spore formers and sulfate reducers by serial dilution produced only one type of colony above the $10^{-3}$ dilution. No colonies formed in any agar shake dilution tubes when the pasteurized culture was transferred to tubes containing butyrate and sulfate but was not reinoculated with a sulfate reducer. Thin sections from the sulfate-reducing consortia show the typical morphology of spores (Fig. 4).

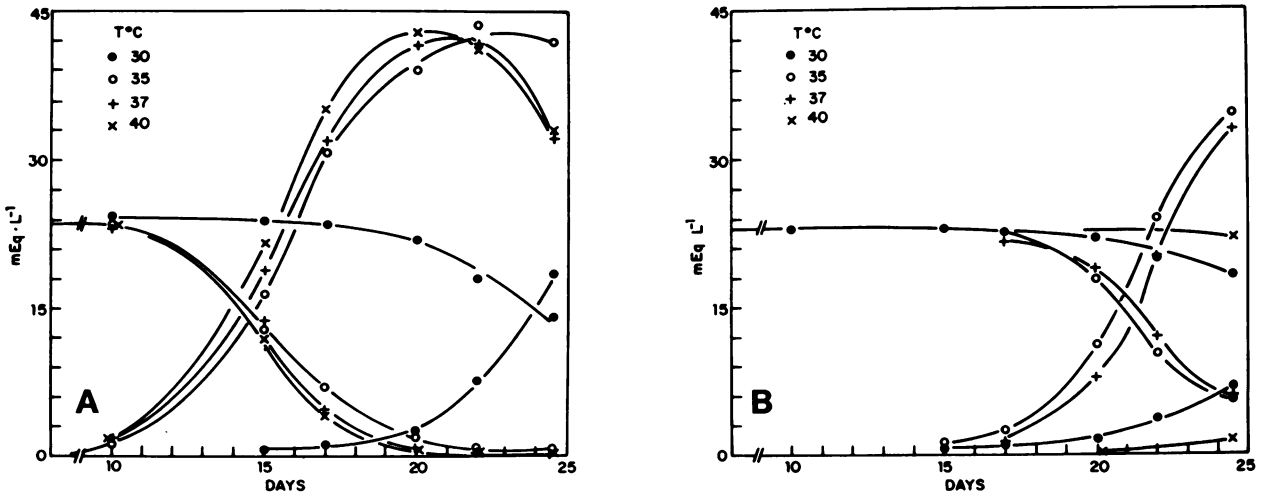


FIG. 2. Pattern of butyrate degradation (decaying curves) and acetate production (ascending curves) as a function of temperature in butyrate enrichments from coal gasification waste (A) and from the Charles River (B). All experiments were carried out in 125-ml stoppered serum bottles containing 50 ml of sulfate-free medium (0.1% [vol/vol] inoculum). Data points represent a single analysis.

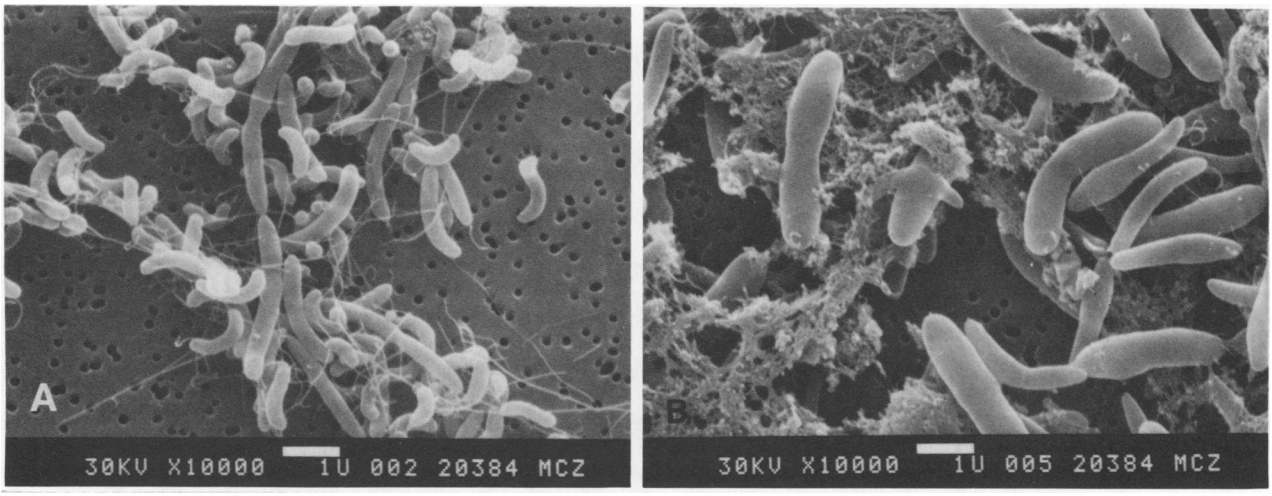


FIG. 3. (A) Scanning electron micrograph from an actively growing culture of a butyrate degrader isolated from coal gasification waste in coculture with *Desulfovibrio* sp. strain CGSO4. The latter was isolated from the same enrichment. Spore formation has not yet occurred. Cells were incubated at 35°C. (B) Scanning electron micrograph of a butyrate degrader isolated from coal gasification waste, in coculture with isolate RotseeSO4 (large bacteria), after spore formation. Cells were incubated at 30°C.

A second spore-forming, butyrate-degrading bacterium may be present in the enrichments from coal gasification waste. Occasionally, in these enrichments we observed a thin non-fluorescent rod. In the $10^{-3}$ dilution of the pasteurized enrichment inoculated with *Desulfovibrio vulgaris* Marburg, a morphologically similar rod with terminal spores was observed. Transfer of this suspension to agar shake tubes produced a second type of colony in the lower dilutions. Efforts are underway to isolate this second bacterium.

**Isolation of a butyrate-degrading consortium from Charles River sediments.** A second spore former, capable of butyrate degradation in consortia with methanogens, was isolated from Charles River sediment samples. The sediments were first incubated exposed to daylight in a Winogradski column. Sediments from the layer that released gas were inoculated into complex medium containing pyruvate, yeast extract, and Casamino Acids. Analyses of the volatile fatty acids showed that acetate and butyrate were the main dissimilatory products. Butyrate was further degraded with a concomitant increase in the concentration of acetate. Acetate was eventually consumed by methanogens and transformed to methane and carbon dioxide.

The pyruvate enrichment was transferred to the defined salts mineral medium supplemented with 25 mM sodium butyrate, the sole energy source. The enrichments were incubated at room temperature. Transfer of the cultures by serial dilution resulted in the enrichment of another spore-forming bacterium growing in clumps with a *Methanobrevibacter*-type fluorescent rod. Also found in smaller numbers was another fluorescent rod resembling *Methanobacterium* sp.

The Charles River enrichment was found to grow optimally at a temperature of 35°C (Fig. 2B). Growth was slower than in the previous enrichment. As in the previous case, a *Methanosarcina* sp. appeared in large numbers after most of the butyrate was degraded to acetate. This strain formed gas vesicles after reaching stationary phase. This enrichment was pasteurized at 80°C for 15 min without killing the spore former, but attempts to pasteurize it at 70°C for 10 min allowed the short methanogen attached to the spore former to survive, producing some unexpected results.

The pasteurized culture was transferred to butyrate-sulfate medium and coinoculated with *Desulfovibrio vulgaris* Madison. Clumps formed as usual, but when observed under the microscope, they were found to be composed of the spore former tightly attached to the short methanogen, with *Desulfovibrio vulgaris* Madison growing in the periphery of the clumps (Fig. 1B). Thus, the short rod seemed to be able to compete for $H_2$ with the sulfate reducer by attaching to the spore former. Pasteurization at 80°C for 15 min eliminated the methanogen from the coculture, as evidenced by the lack of formation of gas bubbles when the culture was transferred to agar-shake tubes and by their formation in tubes inoculated with the culture before pasteurization.

The consortium from coal gasification waste differed markedly from the Charles River consortium. The former grew as an optically dense culture, while the latter grew in

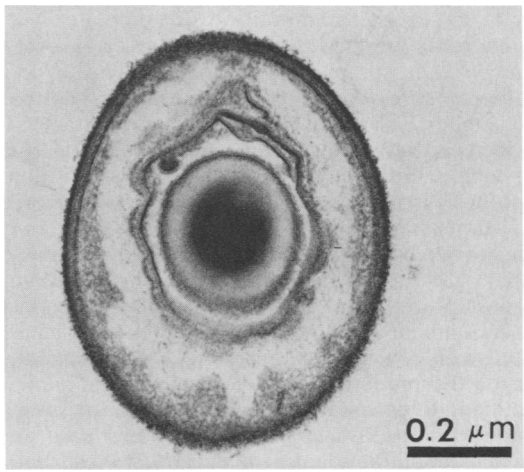


FIG. 4. Transmission electron micrograph showing a cross section of a butyrate-degrading, spore-forming bacterium isolated from coal gasification waste.

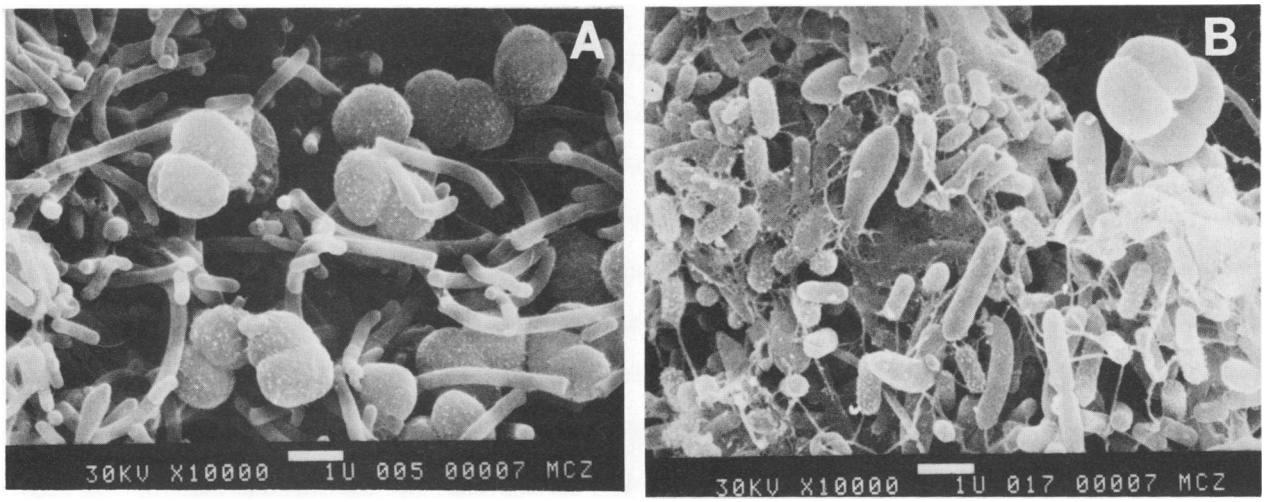


FIG. 5. Scanning electron micrographs of butyrate enrichments in $SO_4^{2-}$-free medium. (A) Enrichment from coal gasification waste. (B) Enrichment from the Charles River.

clumps in an otherwise clear culture. These facts strongly correlated with the observation under scanning electron microscopy of large amounts of extracellular polymer in the enrichment from the Charles River, but not in the enrichment from coal gasification waste (Fig. 5).

## DISCUSSION

The two anaerobic, spore-forming bacteria that we have isolated should be classified within the genus *Clostridium* or *Desulfotomaculum*. *Desulfotomaculum acetoxidans* grows with butyrate as the energy source and sulfate as the electron acceptor (42), but it has not been reported whether it can grow via interspecies hydrogen transfer. None of our isolates were capable of sulfate reduction, and they should presumably be grouped with the clostridia. Formal classification should await further genetic analyses. If alternate means of removing $H_2$ can be found, the ability to pasteurize these spore-forming, proton-reducing bacteria may help to provide pure cultures for physiological studies.

Observations on the selective enrichment of the $H_2$-consuming methanogens in the butyrate enrichments suggest that, as in the competition for acetate between *Methanosarcina* and *Methanothrix* spp. (44, 46), the *Methanospirillum* sp. in coal gasification waste and the *Methanobrevibacter* sp. in the Charles River enrichment rely on higher substrate affinity to outcompete other methanogens. The results also suggest that these methanogens have a lower $\mu_{max}$ than those with lower affinities. Our hypothesis is based on the observation that the predominant $H_2$-consuming methanogen in each of the butyrate enrichments would be outgrown when the cultures were inoculated into media containing nonlimiting amounts of hydrogen (80% in the gas phase).

In an enrichment containing $H_2$-$CO_2$ in the gas phase and inoculated with coal gasification waste, *Methanobacterium* sp. became the predominant methanogen. In a similar enrichment containing sulfate, *Desulfovibrio* sp. became dominant, but methanogens also grew. In the latter case, several methanogens could be identified, but *Methanospirillum* sp. predominated, suggesting that it could grow faster under the lower concentrations of dissolved $H_2$ imposed by the sulfate reducer. Transfer by serial dilution of the $H_2$–$SO_4^{2-}$ enrichment to sulfate-free medium resulted in the predominance of *Methanobacterium* sp. over *Methanospirillum* sp.

Selective enrichment of *Methanospirillum* sp. directly from the butyrate cultures also failed. Transfer to a complex medium containing 80% $H_2$–20% $CO_2$ in the gas phase produced two results: (i) when the medium was incubated at 35°C, *Methanosarcina barkeri* predominated in the culture; (ii) incubation at 45°C, the fluorescent rod that attached to the butyrate degrader dominated. A similar phenomenon was observed in the enrichment from Charles River sediments. The long fluorescent rod predominated over the short one when the enrichments were transferred to a medium containing $H_2$-$CO_2$ in the gas phase.

The levels of hydrogen in our enrichments were below the detection limit of our chromatograph (<1 ppm) indicating that the methanogens quickly removed the $H_2$ produced by the butyrate degraders. Maximum cell density was attained in less than a week when medium inoculated with the enrichments was incubated under an 80% $H_2$ atmosphere, while in the presence of butyrate, maximum cell density was achieved after 3 weeks. The low levels of $H_2$ and the slower rates of growth of the methanogens in the butyrate enrichments imply that the methanogens were $H_2$ limited. Thus, the predominant bacteria under $H_2$-limited conditions were outgrown when $H_2$ was added in excess.

The hypothesis that *Methanospirillum* sp. shows higher affinity for $H_2$ is consistent with other studies of consortia of obligate proton reducers and methanogens. In enrichments from environments in which *Methanospirillum* sp. is found, it invariably appears as the predominant $H_2$-consuming methanogen despite the presence of other competitors for $H_2$ in the same environment (6, 11, 17, 24, 32, 39).

Our observations suggest that attachment may be a strategy used to overcome lower affinities for the growth-limiting substrate. In anaerobic environments, the concentration of $H_2$ is maintained at low levels, and the catabolic processes that remove $H_2$ are kept well below saturation (15, 38); i.e., $H_2$ is found in limiting concentrations. One would expect one $H_2$-consuming bacterium in particular to displace the others, as illustrated for acetate-consuming methanogens (46), yet several $H_2$-oxidizing bacteria could be identified in each of the butyrate enrichments that we have described. Attachment to particular hydrogen producers is one mechanism by

which those bacteria with a lower affinity for hydrogen might compete for the substrate against those bacteria exhibiting a higher affinity. Attachment might allow the hydrogen consumer to scavenge some of the hydrogen before it diffuses into the medium, and attachment may be species specific. These phenomena are illustrated by the selective attachment of the butyrate degrader isolated from the coal gasification waste to specific isolates of sulfate reducers and to *Methanobacterium* sp., but not to the *Methanospirillum* sp. or the *Desulfovibrio* sp. present in the waste. The butyrate degrader from Charles River sediments also showed a strong attachment to *Methanobrevibacter* sp.

Some rough estimates have been made about the proximity of the hydrogen-producing and -consuming bacteria in anaerobic digesters. Gujer and Zehnder (12) calculated that in an anaerobic digester, the bacteria were less than 73 μm apart, based on $H_2$ turnover and diffusion rates. This would imply that on the average most of the $H_2$ is not removed by closely associated consortia. Not many data exist to ascertain the importance of closely associated bacteria in the degradation of particular substrates. However, Boone (6) has postulated that within particle microenvironments the partial pressure of $H_2$ is higher than in the surrounding liquid. This would clearly favor the growth within the particles of bacteria with lower affinity for hydrogen but higher maximum specific growth rates, and those with higher affinity but lower maximum specific growth rates in the surrounding liquid. This model is consistent with our observation that in the butyrate consortium isolated from coal gasification waste *Methanospirillum* sp. grew in suspension, while *Methanobacterium* sp. grew attached in clumps.

The phenomenon of interspecific attachment as the means of competing for $H_2$ could play an important role for the methanogens that use $H_2$ as the energy source and molecules other than $CO_2$ as electron acceptors, e.g., methanol and methylamines. Miller and Wolin (28, 29) have isolated a coccus whose only mode of growth is $H_2$ oxidation-$CH_3OH$ reduction:

$$H_2 + CH_3OH \rightarrow CH_4 + H_2O \quad \Delta G^0 = -112.4 \text{ kJ}$$

*Methanosarcina* sp. strain TM-1, a thermophilic strain, also grows on $H_2$-$CH_3OH$, but not on $H_2$ alone (22). A bacterium with such a highly specialized metabolism faces the problem of simultaneously competing for two substrates that are readily degraded by other bacteria and are thus presumably found under limiting concentrations. The intestinal coccus in particular must compete against the predominant $H_2$-consuming methanogen *Methanobrevibacter smithii* (27, 30). Attachment to a $H_2$-$CH_3OH$ producer, e.g., a pectin degrading bacterium, could overcome the disadvantage of the coccus.

A similar phenomenon could occur during the degradation of trimethylamine. Neill et al. (33) found that in the rumen of unfed sheeps, the $^{14}C$-labeled methyl groups of choline were recovered only in methane. In fed animals, the trimethylamine accumulated without further degradation. This suggests that in the rumen of unfed animals, trimethylamine is degraded via a pathway similar to the one used by the intestinal coccus. Of the methanogenic bacteria isolated from the rumen, only *Methanosarcina barkeri* can grow on $H_2$ and methylamines (34). However, it has been found that *Methanosarcina barkeri* has a much lower affinity for $H_2$ than do the other methanogenic species (19, 35). This strongly suggests that $H_2$ and $(CH_3)_3NH^+$ could come from a choline-degrading bacterium that transfers its metabolites to a closely associated or attached *Methanosarcina* sp. (for stoichiometric reasons there would have to be other sources of hydrogen). In this respect, it is worth noting that despite the apparent low affinity of *Methanosarcina barkeri* for $H_2$, McInerney et al. (25) isolated from the bovine rumen the butyrate-degrading, obligate proton-reducing bacterium *Syntrophomonas wolfei* in coculture with *Methanosarcina barkeri*.

It has been proposed that *Methanobrevibacter ruminantium*, the predominant $H_2$-consuming methanogen in the rumen, does not predominate in butyrate enrichments because of maintenance energy requirements that are too high for growth on the $H_2$ evolving from butyrate oxidation (25). If this is the case, a niche is opened for bacteria such as *Methanosarcina barkeri* to attach to the butyrate degraders and scavenge $H_2$. It is worth noting that at least *Methanosarcina mazei* produces a strongly adhesive polymer on its cell envelope that allows the bacterium to attach to surfaces, and likewise to other bacteria (36). It has also been recently found that in the rumen there are other $H_2$-consuming bacteria morphologically similar to *Methanobrevibacter ruminantium*. One of these isolates withstands starvation and metabolizes $H_2$ at significantly lower concentrations than those found in the rumen (20). Maintenance energy requirements and $H_2$ affinity may not be the only selecting factors in the establishment of consortia.

Our study suggests that individual interspecific bacterial interactions are important in the microbial ecology of anaerobic environments. Efforts are underway to isolate in pure culture the methanogens growing in the consortia and to reestablish them in defined cocultures with the butyrate degraders. This would permit the study of the kinetics of butyrate degradation under defined conditions and the factors that select in favor of particular hydrogen- and acetate-consuming methanogens.

## ACKNOWLEDGMENTS

This work was supported by contract no. NOOO14-81-K-0624 from the Office of Naval Research.

We thank Rafael López for expert technical help. We also thank Susan Houghton, Marine Biological Laboratory, Woods Hole, Mass., and Ed Seling, Museum of Comparative Zoology, Harvard University, for performing the transmission and scanning electron microscopy, respectively. The help of Sai H. Lee in collecting the coal gasification waste samples is greatly appreciated.

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