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Bacterial Strains from Human Feces That Reduce CO_2 to Acetic Acid

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We used dilutions of fecal suspensions from a human volunteer to enrich cultures for bacteria that reduce CO_2 to acetate in the colon. The soluble enrichment substrates used were glucose, methanol, formate, and vanillate, which were used with a gas phase that contained 80% N_2 and 20% CO_2. The gaseous enrichment substrates used were 80% H_2-20% CO_2 and 50% CO-50% CO_2. We isolated three different strains that produced acetate from CO_2. One strain produced acetate from methanol, vanillate, H_2-CO_2, glucose, and other sugars. The other two strains did not form acetate from methanol or vanillate. Both of the latter strains formed acetate from glucose and other sugars, but only one of these strains formed acetate from H_2-CO_2. Both of these strains cometabolized formate. However, none of the enrichment cultures or pure cultures used CO or formate as a substrate for growth. The two strains that produced acetate from H_2 and CO_2 grew slowly when the gases alone were used as substrates, but they rapidly cometabolized H_2 and CO_2 when they were grown with organic substrates. The ability of all of the strains to produce acetate from CO_2 and/or other one-carbon precursors was verified by determining the radioactivity of the methyl and carboxyl groups of the acetate formed after growth with $^{14}CO_2$ or other radioactively labeled one-carbon precursors.

High concentrations of plant polysaccharides (cellulose, hemicellulose, pectin, and starch) are present in the human diet. Cellulose, hemicellulose, and pectin are not digested by host enzymes and pass into the colon, where they are fermented by the cooperative metabolism of a large number of different bacterial species (5, 34). Significant amounts of starch can escape digestion by host small intestine enzymes and can also be fermented in the colon (27, 29). The colonic fermentation of plant polysaccharides produces acetate, propionate, butyrate, and the gases H_2 and CO_2 (5, 18). CH_4 is a major product in some humans (2, 15) who harbor large concentrations of methane-forming *Archaea* strains that use H_2 to reduce CO_2 to CH_4 (20, 30).

The major acid anion product of this fermentation is acetate. On a molar basis, about 3.5 to 4.5 times more acetate than propionate or butyrate is produced. About 10 to 30 g of acetate is produced in the colon daily (26). On the basis of the residual amount of acids per gram of dry fecal matter (31) and assuming daily excretion of 25 g of dry fecal matter, we calculated that about 98% of the acids produced in the colon (6) are absorbed and used by the host. The remaining acids, all of the microbial biomass, and 80% of the H_2 and CH_4 produced are excreted in feces or flatus. Gas absorption into blood and subsequent excretion in breath account for the remaining 20% of the H_2 and CH_4 produced by fermentation (2).

The species responsible for producing almost all colonic CH_4 is *Methanobrevibacter smithii* (20). This organism uses H_2 to reduce CO_2 to CH_4. *Methanosphaera stadtmanae* is less numerous in the colon; it uses H_2 to reduce methanol to CH_4 and cannot produce CH_4 from CO_2 (21). Some people produce essentially no colonic CH_4, while in other people there is constant formation, amounting to several liters a day (2, 22, 30). This great variation in methane production is due to great differences in the concentrations of colonic methanogens. *Methanobrevibacter smithii* concentrations vary from $<10^0$ to 10^{10} cells per g (dry weight) of feces (30). People with 10^8 to 10^{10} *Methanobrevibacter smithii* cells per g (dry weight) of feces produce approximately 0.03 to 3 liters of methane per day and have detectable (>1 ppm) CH_4 in their breath (22).

Each 1 liter of CH_4 produced in the colon requires the production and utilization of 4 liters of H_2. Either much less H_2 is produced when methanogenesis is insignificant or there is an alternative use of the electrons used for reducing CO_2 to CH_4. H_2 is used to reduce CO_2 to acetate in the ceca of rats (24) and the hindguts of termites (4). We hypothesized (34) and later provided evidence that this alternative path of CO_2 reduction is a major human colonic process when the level of methanogenesis is negligible (14). Acetate formation from CO_2 would also explain the origin of the large amounts of colonic acetate produced in nonmethanogenic humans (34).

Although *Methanobrevibacter smithii* is responsible for reducing CO_2 to CH_4 in the human colon (20, 30), we cannot specify the colonic bacteria responsible for reducing CO_2 to acetate. Many different bacterial genera and species reside in the colon. The ability of most of these species to reduce CO_2 to acetate has not been investigated. Although *Peptostreptococcus productus* isolated from sewage sludge (17) produced acetate from H_2-CO_2 and CO, strains isolated from human feces did not grow on CO (17). Most-probable-number estimates of bacteria in human feces showed that only 1.1×10^5 bacteria per g (wet weight) of feces grew on CO (17). A strain of *P. productus* that grew on CO was also isolated from sewage sludge by Geerligs et al. (9). Geerligs et al. reported that the type strain of *P. productus* (isolated from human feces) did not grow on CO (9).

Another species, *Eubacterium limosum* (formerly called *Butyribacterium rettgeri*), produced acetate from H_2-CO_2 and methanol (10). Barker et al. showed that *B. rettgeri* produced acetate from CO_2 during carbohydrate fermentation (1). *E. limosum* is not found in many people, and when

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it is present, its concentrations in feces are not exceptionally high (7a). The bacterial species isolated from termite guts (4) or rumina (11) that form acetate from CO_2 have not been found in human feces.

In this study, we performed experiments designed to selectively isolate and characterize bacteria that reduce CO_2 to acetate in the human colon. These experiments resulted in the isolation of several different organisms that reduce CO_2 to acetate. In this paper we describe the isolation procedures used, the distinctive characteristics of the isolates, and the results of radioisotope experiments that verified the ability of the organisms to form acetate from CO_2.

MATERIALS AND METHODS

Fecal suspensions. Fecal samples were provided by a healthy adult with no history of diagnosed large bowel disease. The volunteer, who does not harbor significant concentrations of fecal methanogens, was subject 1 in previous studies of fecal microbial community fermentation (31, 32). Human fecal fermentation protocols were reviewed and approved yearly by the New York State Department of Health Institutional Review Board.

Suspensions (10%, wt/vol) of feces were prepared in an anaerobic dilution solution as described previously (31). The suspensions were kept at 4°C and used within 24 h of collection. Amounts of fecal dry matter were determined as described previously (31).

Anaerobic methods. Anaerobic conditions were maintained by using the serum bottle modification of the Hungate technique (19). The basal medium used for enrichment cultures, isolation, and growth of pure cultures contained (per liter): $NaHCO_3$, 7.5 g; K_2HPO_4, 0.3 g; KH_2PO_4, 0.3 g; $(NH_4)_2SO_4$, 0.3 g; NH_4Cl, 1 g; NaCl, 0.61 g; $MgSO_4 \cdot 7H_2O$, 0.12 g; $CaCl_2 \cdot 2H_2O$, 80 mg; $MgSO_4 \cdot 7H_2O$, 30 mg; $MnSO_4 \cdot H_2O$, 4.5 mg; $FeSO_4 \cdot 7H_2O$, 3.0 mg; $CoSO_4 \cdot 7H_2O$, 1.8 mg; $ZnSO_4 \cdot 7H_2O$, 1.8 mg; $CuSO_4 \cdot 5H_2O$, 100 μg; $AlK(SO_4) \cdot 12H_2O$, 180 μg; $Na_2MoO_4 \cdot 2H_2O$, 100 μg; H_3BO_3, 100 μg; Na_2SeO_4, 1.9 mg; $NiCl_2 \cdot 6H_2O$, 92 μg; nitrilotriacetic acid, 15 mg; thiamine-HCl, 2.0 mg; D-pantothenic acid, 2.0 mg; nicotinamide, 2.0 mg; riboflavin, 2.0 mg; pyridoxine-HCl, 2.0 mg; biotin, 10.0 mg; cyanocobalamin, 20 μg; *p*-aminobenzoic acid, 100 μg; folic acid, 50 μg; cysteine-HCl · H_2O, 0.5 g; rumen fluid, 100 ml; sodium formate, 0.5 g; and yeast extract, 2 or 0.4 g as indicated below. Resazurin (1 mg/liter) was added as an oxidation-reduction potential indicator.

The medium was prepared and autoclaved under 100% CO_2. The medium was aseptically reduced by adding a sterile solution containing 1.25% cysteine and 1.25% sodium sulfide (30 μl/ml of medium). After inoculation, culture tubes were aseptically regassed with specific gas mixtures as indicated below. All gases were passed through a heated column containing copper filings to remove trace amounts of oxygen. All liquid cultures were incubated on a rotator at 37°C. The basal medium described above containing 2% agar was used to prepare agar roll tubes for isolation of colonies of bacteria that reduce CO_2 to acetate from enrichment cultures. The roll tubes were incubated statically at 37°C.

Enrichment cultures. Bromoethanesulfonate (final concentration, 4 mM) was aseptically added to inhibit growth of methanogens. Organic substrates were prepared in boiled and cooled distilled water and were sterilized by passage through a 0.2-μm-pore-size filter as they were transferred into a sterile serum bottle under N_2. When organic substrates were added, their concentrations in enrichment cultures were as follows: glucose, 0.25%; methanol, 0.25%; sodium formate, 0.3%; and sodium vanillate, 1.0%. The gas phase in these cultures consisted of 103 kPa of 80% N_2–20% CO_2. The gaseous substrate mixtures used for enrichment cultures were 206 kPa of 80% H_2–20% CO_2 and 206 kPa of 50% CO–50% CO_2. A substrate-free control prepared with 103 kPa of 80% N_2–20% CO_2 and all substrate-containing media were inoculated by adding 0.5-ml portions of selected serial 10-fold dilutions to 10-ml portions of medium in tubes (18 by 150 mm) with a serum bottle finish. Growth was measured by determining the optical density at 660 nm.

Analytical methods. The concentrations of glucose and fermentation products were determined using the high-pressure liquid chromatographic (HPLC) procedures described by Ehrlich et al. (7). The injection volume was 20 μl, and the column was an Aminex HPX-87H column (Bio-Rad Laboratories, Rockville Center, N.Y.); elution was isocratic with 0.013 N H_2SO_4 at 35°C. The flow rate was 0.5 ml · min^{-1} at a pressure of 63 kg/cm^2. Eluting compounds were detected by their refractive indexes and were identified and quantified by using a Chromatopac model C-R3A integrator-data processor (Shimadzu, Columbia, Md.) and the absolute calibration curve method. Hydrogen and CO_2 concentrations were determined by gas chromatography as described by Pavlostathis et al. (23).

^{14}C-labeling experiments. Radioactive solutions of sodium bicarbonate, sodium formate, and methanol (New England Nuclear, Boston, Mass.) were prepared in boiled and cooled distilled water, filter sterilized (pore size, 0.2 μm), and stored under N_2 at −20°C. Radioactive substrates were added aseptically to reduced, sterile media. After inoculation, the tubes were pressurized to 206 kPa with a gas phase and incubated with rotation at 37°C. After growth, the cultures were acidified with 50 μl of 5 N H_2SO_4 per ml of culture. The total volume of gas in a culture was determined by adding the volume of gas displaced into an inserted hypodermic syringe to the predetermined gas headspace volume of the culture tube. After the amounts of H_2 and CO_2 were determined, portions were removed for radioactivity measurements.

After all gas measurements, each culture tube was transferred to a fume hood, the headspace was gassed with N_2 for 10 min, and the culture liquid was then sparged with CO_2 for 10 min. The culture was centrifuged at 1,397 × *g* for 10 min. The supernatant was passed through a 0.22-μm-pore-size filter and stored at −20°C until further analysis.

Radioactivity of CO_2 and acetate carbon atoms. Duplicate 5-ml portions of the gas phases of acidified cultures were injected into stoppered and sealed serum bottles containing 4.0 ml of scintillation grade phenethylamine (Eastman Kodak, Rochester, N.Y.) and an N_2 atmosphere. The gas and liquid contents were mixed without removing the syringe and needle. After incubation at room temperature for 10 min, the amount of unabsorbed gas was measured by the displacement of a hypodermic syringe plunger, and the value was used to calculate the amount of CO_2 absorbed into phenethylamine. Acetate was purified from 100 μl of acidified fermentation supernatant by the HPLC procedure used to determine the concentrations of glucose and fermentation products. The acetate fraction detected by the refractive index detector was collected in ca. 1.5 ml of eluant. A portion (0.1 ml) was removed to determine the amount of supernatant radioactivity recovered in the acetate fraction. The average level of recovery of authentic ^{14}C-labeled acetate was 80 to 90%.

The pH of the ^{14}C-labeled acetate fraction was adjusted to

8.0 with 0.3 N NaOH, 40 μmol of nonradioactive sodium acetate was added, and the preparation was dried overnight in a 90°C oven. The acetate carboxyl and methyl groups were sequentially oxidized to CO_2 by the Schmidt procedure described by Fuchs et al. (8). The CO_2 in the excess headspace volume (ca. 10 to 15 ml) of the reaction vessel was quantitatively transferred to a 100-ml serum bottle containing 3 ml of phenethylamine and 20 glass beads (diameter, 3 mm). Schmidt degradation of authentic [U-^{14}C]acetate yielded equivalent radioactivity from each of the carbon atoms and a combined level of recovery of 96% of the initial radioactivity.

Radioactivity measurements. The amounts of radioactivity in stock solutions, acidified culture supernatants, and CO_2 trapped in phenethylamine were determined in Aquasol (New England Nuclear) by using a scintillation counter (model 1217 Rackbeta; LKB Wallac, Turku, Finland). The radioactivity values were corrected for quenching by the external standard method.

RESULTS

Enrichment cultures. We used 80% H_2–20% CO_2, 50% CO–50% CO_2, formate, methanol, and vanillate to enrich cultures for bacteria that reduce CO_2 to acetate. We also used glucose as a general enrichment substrate. The basal medium contained bromoethanesulfonate to eliminate the possibility of growth of methanogens. The inoculum used was 0.5 ml of a 1:1,000 dilution of a fecal suspension. Significant glucose-dependent growth was observed after 24 h. Although H_2 and CO_2 did not support growth, there was slow utilization of H_2. Little H_2 had disappeared after 8 days, but almost all H_2 disappeared after 18 days. No growth or utilization of the enrichment substrate added was observed with CO or formate.

Methanol and vanillate enrichment cultures grew, but it took about 6 to 7 days for the optical density to increase above the level of the basal medium control containing no added substrate. Methanol-to-methanol and vanillate-to-vanillate transfers were made from the enrichment cultures into sterile enrichment medium. Duplicate transfers of each substrate enrichment culture were incubated in 80% N_2–20% CO_2 and 80% H_2–20% CO_2 atmospheres. Good substrate-dependent growth was obtained within 1 to 2 days with all transfers. Interestingly, H_2 was rapidly used by the bacteria transferred from the methanol and vanillate enrichment cultures. Large amounts of acetate (41 to 43 mM) were formed in methanol and vanillate enrichment cultures incubated in an 80% N_2–20% CO_2 atmosphere, and higher amounts (79 to 96 mM) were produced when 80% H_2–20% CO_2 was the gas phase. Large amounts of ethanol and small amounts of propionate were also found in all preparations transferred from the methanol and vanillate enrichment cultures.

Isolation of a vanillate-using strain. To improve the selectivity of the medium, we first eliminated substantial vanillate-independent growth and fermentation by preparations transferred from the vanillate enrichment culture by lowering the yeast extract concentration to 0.04%. The transferred cultures grew to an optical density of more than 2 within 24 to 48 h with vanillate, whereas the optical density was ca. 0.2 without vanillate. Sequential transfer preparations were subsequently grown in the vanillate-containing medium supplemented with 0.04% yeast extract. Dilutions of a transferred preparation were inoculated into agar roll tubes with medium containing 0.04% yeast extract and 1% vanillate and incubated with 101 kPa of 80% H_2–20% CO_2.

TABLE 1. Radioactivity of acetate formed by strain CS1Van grown with $^{14}CH_3OH$ or $^{14}CO_2$[a]

^{14}C-labeled substrate	Total amt of acetate (μmol)	dpm/μmol		
		Acetate	Acetate methyl group	Acetate carboxyl group
Methanol	161	1,710	1,327	238
Carbon dioxide	422	2,603	209	2,042

[a] The organism was grown with 202 mM methanol under an 80% N_2–20% CO_2 atmosphere with different radioactive substrates.

Isolated colonies contained gram-positive, short, plump rods with rounded ends that used vanillate as a substrate for growth. Strain CS1Van grew with methanol, but not with CO, H_2, or formate added as the substrate. It did not require added H_2 for growth in the presence of vanillate or methanol. However, added H_2, but not added formate, was rapidly used when the isolate was grown with vanillate.

A large number of other substrates were tested (all at a final concentration of 0.5%), and growth and fermentation were obtained with adonitol, fructose, glucose, lactate, maltose, mannitol, pyruvate, ribose, and xylose; poor growth occurred with pectin and arabinose. The major product of fermentation of all substrates was acetate. Butyrate was a significant product with some substrates (e.g., fructose and vanillate) but not with other substrates (e.g., glucose and methanol). The substrates that were not fermented were alanine, Avicel, carboxymethyl cellulose, dextrin, dulcitol, ferulic acid, fumarate, galactose, glycerol, inositol, malate, melezitose, melibiose, raffinose, rhamnose, salicin, serine, starch, succinate, trehalose, and xylan. The fermentations yielded optical density values equal to or greater than five times the optical density of a control without added substrate after 24 to 72 h. Cultures which exhibited no fermentation had an optical density equal to that of the same control after 192 h. Poor fermentation produced optical density values that were two to four times that of the same control after 192 h. The highest optical density value of the control without substrate was 0.25. The results were verified by an HPLC analysis of the products.

When methanol and CO_2 were the substrates, acetate was labeled when either radioactive methanol or radioactive CO_2 was supplied (Table 1). The specific activities of acetate and its methyl and carboxyl groups indicated that most of the methyl group was formed from methanol and most of the carboxyl group was formed from CO_2 (Table 1).

Glucose- and methanol-using strains that reduce CO_2 to acetate. We repeated the dilution-enrichment series with 0.5-ml inocula prepared from a 10^{-6} dilution of a fresh fecal

TABLE 2. Substrates and growth of strain CS3Glu

Addition	Optical density at 660 nm	
	N_2-CO_2	H_2-CO_2
Glucose (0.05%)	0.95 (21)[a]	1.90 (45)
None	0.23 (21)	0.50 (45)
Methanol (0.25%)	0.19 (22)	0.49 (48)

[a] The numbers in parentheses are the numbers of hours required to reach the maximum optical density. All H_2 (1.1 mmol) was used when the maximum optical density was measured.

TABLE 3. Fermentation of glucose plus formate by strain CS3Glu[a]

Compound	Total amt used or formed (μmol)[b]	μmol/100 μmol of glucose	Total amt of carbon (μmol)	Oxidation state	Oxidation level
Substrates					
Glucose	264.3	100.0	600.0	0	0.0
CO_2[c]	29.9	11.3	11.3	−2	−22.6
Formate	395.9	149.8	149.8	−1	−149.8
Total			761.1		−172.4
Products					
Succinate	59.8	22.6	90.5	1	22.6
Acetate	783.2	296.4	592.7	0	0.0
Lactate	1.4	0.5	1.6	0	0.0
CO_2	198.0	74.9	74.9	2	149.8
Total			759.7		172.4

[a] Cultures were grown with the total amounts of glucose and formate indicated with an 80% N_2–20% CO_2 atmosphere at 37°C.
[b] Amount of substrate used or product formed.
[c] The amount of CO_2 was calculated by using the following equations: $3C_6H_{12}O_6 + 4HCO_2H \rightarrow 10CH_3COOH + 2CO_2 + 2H_2O$ and $3C_6H_{12}O_6 + 2CO_2 \rightarrow 4HOOC(CH_2)_2COOH + 2CH_3COOH + 2H_2O$.

suspension. The substrates used were methanol, vanillate, glucose, and formate at the concentrations used in the first experiment. Medium supplemented with 0.04% yeast extract was used, and all enrichment cultures had 80% H_2–20% CO_2 in the gas phase. The no-substrate control was incubated in an 80% N_2–20% CO_2 atmosphere to examine substrate-independent growth. Again, the only substrates that yielded significant growth were glucose, methanol, and vanillate. We isolated a gram-positive, short, plump rod (strain CS3MeOH) from the methanol enrichment culture that grew well with methanol under N_2 and also used H_2 rapidly when it was grown with methanol and with 80% H_2–20% CO_2 in the gas phase. It did not grow with H_2 alone. We have not yet determined whether this organism is the same organism that we isolated from the first vanillate enrichment cultures (see above).

Rapid use of H_2 occurred in the glucose enrichment culture. Cultures transferred to media containing a lower concentration of glucose (0.05%) grew and used H_2. We isolated colonies on 0.05% glucose agar medium with 80% H_2–20% CO_2 in the gas phase. We isolated a strain that produced acetate from CO_2 (strain CS3Glu), and this strain differed from the organisms isolated previously from vanillate and methanol enrichment cultures. This organism was a gram-positive coccobacillus that occurred singly, in pairs, and in short chains. It grew with 80% H_2–20% CO_2 alone or with glucose and 80% N_2–20% CO_2, but it did not use methanol (Table 2). Although it did not grow with formate as a sole substrate, CS3Glu cometabolized formate and glucose to form large amounts of acetate and small amounts of succinate (Table 3). Radioisotope experiments (data not shown) showed that strain CS3Glu incorporated radioactive carbon dioxide into both carbon atoms of acetate.

Second glucose-using strain that formed acetate from CO_2. We repeated the enrichment culture experiments that yielded isolate CS3Glu with a fresh fecal specimen to try to isolate a similar glucose- and H_2-using organism from higher dilutions of fecal suspensions. We used 0.5-ml inocula from 10^{-6} to 10^{-10} dilutions of the fecal suspension from the same subject in the same medium containing a low concentration of yeast extract, and 0.25% glucose as the substrate. The gas phase was 80% H_2–20% CO_2. In contrast to the previous dilution-enrichment series, poor or no H_2 utilization was found in the cultures prepared with inocula from the 10^{-6} to 10^{-10} dilutions. However, inocula from the 10^{-6} to the 10^{-9} dilutions, but not from the 10^{-10} dilution, produced cultures that used glucose and the 0.04% formate added to the medium and formed high concentrations of acetate. The inoculum from the 10^{-10} dilution produced cultures that used glucose and formed considerable amounts of ethanol with a significant net production of formate.

Attempts to isolate pure cultures from the 10^{-9} dilution by using agar roll tubes containing the same medium used for the enrichment cultures (i.e., low yeast extract concentration, 0.25% glucose, an 80% H_2–20% CO_2 gas phase) were unsuccessful. All colonies picked yielded a gram-positive coccus that produced acetate, ethanol, and formate. Although spores were not detected by phase-contrast micros-

TABLE 4. Fermentation of glucose plus formate by strain CS7H[a]

Compound	Total amt used or formed (μmol)[b]	μmol/100 μmol of glucose	Total amt of carbon (μmol)	Oxidation state	Oxidation level
Substrates					
Glucose	128.6	100.0	600.0	0	0.0
Formate	607.8	472.5	472.5	−1	−472.5
Total			1,072.5		−472.5
Products					
Acetate	538.2	418.4	836.8	0	0.0
CO_2[c]	303.9	236.2	236.2	2	472.5
Total			1,073.0		472.5

[a] Cultures were grown with the total amounts of glucose and formate indicated with an 80% N_2–20% CO_2 atmosphere as the gas phase.
[b] Amount of substrate used or product formed.
[c] The amount of CO_2 was calculated by using the following equation: $3C_6H_{12}O_6 + 4HCO_2H \rightarrow 10CH_3COOH + 2CO_2 + 2H_2O$.

TABLE 5. Radioactivity of acetate formed by strain CS7H grown with glucose and $^{14}CO_2$ or [^{14}C]formate

^{14}C-labeled substrate	Total amt of acetate (μmol)	dpm/μmol		
		Acetate	Acetate methyl group	Acetate carboxyl group
Formate[a]	639	1,421	1,052	68
Carbon dioxide[b]	495	1,563	765	809

[a] The organism was grown with 23 mM glucose under an 80% N_2–20% CO_2 atmosphere with 21 mM radioactive sodium formate.

[b] The organism was grown in the absence of formate with 23 mM glucose under an 80% N_2–20% CO_2 atmosphere with radioactive carbon dioxide.

copy, we heated transfers of the enrichment culture originally inoculated with the 10^{-9} dilution of feces at 80°C for 10 min. Roll tubes were prepared with medium containing the low yeast extract concentration, 0.25% glucose, and an 80% H_2–20% CO_2 gas phase by using dilutions of the heated culture. A strain that made acetate from CO_2 was isolated (strain CS7H). This organism was a gram-positive, short, plump rod that survived heating at 80°C but not heating at 100°C for 10 min. We did not detect spores in the culture by phase-contrast microscopy. This strain required CO_2 for growth, and did not use H_2 and CO_2 as sole substrates for growth. It used glucose and cometabolized formate but did not grow with formate as the sole substrate and did not require formate for growth on glucose.

Acetate was the sole product of fermentation of 0.25% glucose and 0.5% formate (Table 4). Separate radioisotope experiments were performed to measure incorporation of CO_2 and formate into acetate. CO_2 was incorporated equally into both carbon atoms of acetate, while formate was incorporated into the methyl group (Table 5). No radioactivity from formate was found in CO_2. Fermentation tests were performed as described above for strain CS1Van. Isolate CS7H did not ferment adonitol, alanine, Avicel, carboxymethyl cellulose, dextrin, dulcitol, ferulic acid, fumarate, glycerol, inositol, lactate, malate, pectin, pyruvate, rhamnose, serine, starch, succinate, or xylan. It rapidly fermented arabinose, fructose, galactose, glucose, lactate, lactose, maltose, mannitol, melezitose, melibiose, raffinose, salicin, stachyose, sucrose, trehalose, and xylose.

DISCUSSION

The isolation, characterization, and estimation of the significance of bacteria that reduce CO_2 to acetate in the human colon present a much greater challenge than comparable studies of methanogens. The same species of H_2-using methanogen is found in a large number of individuals (20, 22, 30). However, the colon of the donor of the fecal specimens which we examined contains at least three different types of bacteria that form acetate from CO_2 (Table 6). None of these seems to be identical to any of the previously described species of bacteria that use the Wood-Ljungdahl pathway to produce acetate from CO_2 (4, 9, 11, 13, 16, 35), although the isolates obtained from the methanol and vanillate enrichment cultures may be related to *E. limosum* (1, 10). There probably are additional species of colonic bacteria that produce acetate from CO_2 that have not been isolated.

Unlike the situation with H_2-using methanogens, selective isolation and enumeration of bacteria that form acetate from CO_2 are not straightforward. Antibiotics can be used to selectively grow methanogens because many antibiotics that kill members of the *Bacteria* do not kill members of the *Archaea*. In contrast, there is no obvious selective agent or substrate that can be used to enumerate and isolate bacteria that reduce CO_2 to acetate. None of the enrichment cultures or pure cultures used CO or formate as a substrate for growth. When H_2 and CO_2 were used as sole substrates, the growth of enrichment cultures was slow and poor. None of the isolates grew well with H_2 and CO_2 as sole substrates. Isolate CS7H did not use H_2 to reduce CO_2 to acetate even when it was growing with glucose. Isolate CS1Van did not grow with H_2 and CO_2 but cometabolized these gases when it was grown with vanillate or methanol. Isolate CS3Glu grew poorly with H_2 and CO_2 alone, but cometabolized the gases rapidly when it was grown with glucose. Similar mixotrophic growth with H_2-CO_2 has been demonstrated with bacteria isolated from the termite gut that form acetate from CO_2 (3).

The importance of evaluating the relative contribution of the reduction of CO_2 to acetate to the overall colonic fermentation is underscored by the results of recent studies of the physiological effects of the fermentation acids. Acetate infused into the colon increases blood cholesterol levels, whereas infused propionate is gluconeogenic (33). Propi-

TABLE 6. Characteristics of bacteria in human feces that reduce CO_2 to acetate

Strain	Amt of fecal dry matter used for isolation (g)	Morphology	Acetate produced from:	Gram stain reaction	Labeling in acetate from:			Other
					$^{14}CO_2$	$H^{14}CO_2H$	$^{14}CH_3OH$	
CS1Van	5×10^{-4}	Short plump rod	Methanol, vanillate, H_2-CO_2, glucose, other sugars	+	COOH[a]	ND[b]	CH_3[a]	Grows slowly with H_2 but uses H_2 rapidly when grown with vanillate or methanol
CS3Glu	5×10^{-7}	Coccobacillus	Glucose, other sugars, H_2-CO_2	+	COOH, CH_3[c]	ND	ND	Grows slowly with H_2 but uses H_2 rapidly when grown with glucose
CS7H	5×10^{-10}	Short plump rod	Glucose, other sugars	+	COOH, CH_3[d]	CH_3[e]	ND	Survives heating at 80°C for 10 min

[a] Grown with methanol and N_2-CO_2.

[b] ND, not done.

[c] Grown with glucose with N_2-CO_2.

[d] Grown with glucose and N_2-CO_2 but without formate.

[e] Grown with glucose, N_2-CO_2, and formate.

onate infusion depresses cholesterol synthesis from acetate. The conversion of propionate to glycogen in the liver may be one of the benefits of the high-fiber diets recommended for diabetics. Colonic infusion of acetate, propionate, and butyrate decreased colonic bleeding in patients with diversion colitis (26). Butyrate is used by colonic epithelial cells as an energy source (25). This compound has powerful properties as a cell-differentiating agent (12). Low concentrations of butyrate cause differentiation of mammalian cells, including human colon carcinoma cells (28).

Our results suggest that reduction of CO_2 to acetate in the colon depends on bacteria that use primarily organic substrates as sources of carbon and energy. These bacteria also produce acetate by cometabolizing H_2-CO_2 or formate when they grow on organic substrates. Additional studies will be necessary to determine whether the bacteria that reduce CO_2 to acetate that we isolated and/or other bacteria contribute significantly to the regulation of the production of the colonic fermentation acids.

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