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Growth Substrate Effects on Acetate and Methanol Catabolism in *Methanosarcina* sp. Strain TM-1

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When *Methanosarcina* sp. strain TM-1 is grown in medium in which both methanol and acetate are present, growth is biphasic, with methanol used as the primary catabolic substrate during the first phase. To better understand this phenomenon, we grew cells on methanol or on acetate or on both and examined the abilities of anaerobically washed cells to catabolize these substrates. Washed acetate-grown cells incubated with 10 mM acetate, 10 mM methanol, or both substrates together produced methane at initial rates of 325, 3, and 315 nmol min^{-1} mg of $protein^{-1}$, respectively. Although the initial rate of methanogenesis from both substrates was nearly identical to the rate for acetate alone, after several hours of incubation the rate was greater for cells provided with both substrates. Studies with ^{14}C-labeled methanol indicated that methanol was catabolized to methane at increasing rates by acetate-grown cells in a manner reminiscent of an induction curve, but only when cells were provided with acetate as a cosubstrate. Acetate was presumably providing energy and carbon for induction of methanol-catabolic enzymes. Methanol-grown cells showed a pattern of substrate utilization significantly different from that of acetate-grown cells, producing methane from 10 mM acetate, 10 mM methanol, or both substrates at initial rates of 10, 280, and 450 nmol min^{-1} mg of $protein^{-1}$, respectively. There was significant oxidation of the methyl group of acetate during metabolism of both substrates. Cells grown on methanol-acetate and harvested before methanol depletion (methanol phase) showed catabolic patterns nearly identical to those of methanol-grown cells, including a low rate of methanogenesis from acetate. Cells harvested from methanol-acetate cultures in the acetate phase were capable of significant methanogenesis from either methanol or acetate alone, and the rate from both substrates together was nearly equal to the sum of the rates for the single substrates. When both 10 mM methanol and 10 mM acetate were presented to the acetate-phase cells, there was a preference for the methanol. These results are consistent with a model for regulation in *Methanosarcina* sp. strain TM-1 in which methanol represses acetate catabolism while methanol catabolism is inducible.

The methanogenic bacteria are characterized by extreme catabolic specialization, generally producing only CH_4 and CO_2 as metabolic end products (1, 14, 26). Many methanogens, such as *Methanobacterium thermoautotrophicum*, use only H_2 and CO_2 for methanogenesis, while others, such as *Methanobacterium formicicum*, also use formate. *Methanothrix soehngenii* uses only acetate as a substrate for methanogenesis (8). Of the known methanogens, *Methanosarcina* is the genus with the greatest catabolic versatility, with most strains capable of using H_2-CO_2, methanol, methylamines, or acetate as methanogenic substrates (1, 14, 20, 21, 26). This versatility makes *Methanosarcina* spp. good candidates for the study of catabolic regulation.

Several investigators (8, 9, 12, 18, 19, 24, 28) have found that when various *Methanosarcina* cultures are presented with both methanol and acetate in the growth medium, growth is biphasic. Methanol is initially the preferred substrate for methanogenesis (methanol phase), and once methanol is depleted, acetate is used extensively for methanogenesis (acetate phase). H_2-CO_2 (6, 25) and methylamines (4) are also generally preferred over acetate as methanogenic substrates. During catabolism of a preferred substrate, some methanogenesis from acetate can occur, and acetate can also be assimilated into cell carbon or oxidized to CO_2 (4, 6, 12, 20, 24, 28). Cultures of *Methanosarcina* spp. previously grown on a preferred substrate, such as methanol or H_2-CO_2, and then transferred onto growth medium in which acetate is the sole methanogenic substrate often show lags of up to several weeks before methanogenesis and growth commence (14, 15, 20, 24, 25).

The regulatory mechanism(s) involved in this substrate preference is presently unknown, and possibilities include (i) out-competition by the preferred substrate for an enzyme-binding site or common pathway intermediate such as coenzyme M, (ii) inhibition of acetate-catabolizing enzymes, and (iii) repression of acetate-catabolizing enzymes. It has also been suggested that some *Methanosarcina* cultures, especially those cultured for long periods on substrates other than acetate, must become "acetate adapted", implying that a heritable change must occur to allow rapid growth on acetate (12, 20, 24, 25). In general, little is known about regulatory mechanisms in methanogens or other archaebacteria.

Repression of acetate catabolism would mean that one or more gene products involved in acetate breakdown would be found in significantly decreased levels in cells grown in the presence of methanol. Baresi and Wolfe (3) assayed methylcoenzyme M methylreductase, hydrogenase, coenzyme M, and coenzyme F_{420} in cells of *Methanosarcina barkeri* 227 grown on H_2-CO_2, methanol, or acetate and found little difference in their levels. Kuhn et al. (13) found no difference in the levels of cytochrome *b* in cells grown on different substrates. Some positive evidence for induction or repression of acetate catabolism has accrued. Baresi (2) found that when an acetate-grown culture of *Methanosarcina barkeri* 227 was transferred several times on H_2-CO_2, cell lysates lost the ability to cleave acetate.

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Krzycki et al. (12) found that acetate-grown cells of *Methanosarcina barkeri* MS had fivefold-higher levels of carbon monoxide dehydrogenase, an enzyme likely to be involved in acetate cleavage, than did methanol or H_2-CO_2-grown cells. In other studies concerning potential catabolic regulatory systems in methanogens, Schauer and Ferry (17) found about twofold more formate dehydrogenase and coenzyme F_{420} in formate-grown cells of *Methanobacterium formicicum* than in H_2-CO_2-grown cells and similar levels of hydrogenase in cells grown on either substrate. Naumann et al. (16) found trimethylamine:HS-coenzyme M methyltransferase activity to be detectable only in cells of *Methanosarcina barkeri* which had been grown in the presence of trimethylamine, indicating an inducible enzyme system.

We have been investigating the nature of the regulation of methanol and acetate catabolism in the thermophile *Methanosarcina* sp. strain TM-1 (28). Sowers et al. (22) recently showed that *Methanosarcina* sp. strain TM-1 has 14 to 34% DNA homology to other *Methanosarcina* strains tested and 86 to 89% rRNA homology, and it is proposed that strain TM-1 will represent a new species named "*Methanosarcina thermophila*" (S. H. Zinder, K. Sowers, and J. G. Ferry, Int. J. Syst. Bacteriol., in press). *Methanosarcina* sp. strain TM-1 showed biphasic growth on a methanol-acetate mixture (28). During phase 1, methanol accounted for over 90% of the methanogenesis, while roughly equal portions of the methyl group of acetate metabolized were converted to CH_4, oxidized to CO_2, and incorporated into cell material (20, 28). The culture rapidly shifted to utilizing acetate as a methanogenic substrate after methanol depletion. We report here that the ability of cells of *Methanosarcina* sp. strain TM-1 to catabolize acetate or methanol or both greatly depends upon which substrate they were grown on.

MATERIALS AND METHODS

Microbial strains. *Methanosarcina* sp. strain TM-1 (28) was used in all the experiments described below. The culture was originally maintained by frequent transfer into liquid methanol-acetate medium (28). For the present studies, the culture was divided into two subcultures that were transferred in growth medium with 40 mM sodium acetate or 24 mM methanol, respectively. These cultures were transferred every 1 to 3 weeks, had been transferred at least 10 times in these growth media before use in the experiments, and in most cases had been transferred for more than 1 year.

Culture media and conditions. The basal medium for the culture of *Methanosarcina* sp. strain TM-1 contained (grams per liter): NH_4Cl, 1.0; K_2HPO_4, 0.4; $MgCl_2 \cdot 6H_2O$, 0.1; yeast extract (Difco Laboratories, Detroit, Mich.), 0.1; resazurin, 0.001; and trace metal solution (as in reference 26 except with the addition of 0.02 g of $NiCl_2 \cdot 6H_2O$ per liter), 10 ml. After the basal medium was boiled under N_2 (scrubbed of trace oxygen by hot copper coils), neutralized cysteine hydrochloride was added to a concentration of 0.5 g/liter, and the medium was boiled further until the resazurin was reduced. This medium was then dispensed in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) in 50-ml quantities into 118-ml serum bottles, which were then sealed with butyl rubber stoppers (Bellco Glass, Inc., Vineland, N.J.) and aluminum crimps (1). After the vials were autoclaved, the vial headspaces were flushed with filter-sterilized 70% N_2–30% CO_2 (Matheson Gas Products, Inc., Joliet, Ill.), and the following additions were made to the medium (grams per liter): $NaHCO_3$, 1.0; $CaCl_2 \cdot 9H_2O$, 0.1; $Na_2S \cdot 9H_2O$, 0.1. The methanogenic substrate, 40 mM sodium acetate or 24 mM methanol or both, was then added. Gas chromatographic analysis (27) showed that the methanol growth medium contained less than 0.03 mM acetate. The pH of the growth medium was 6.5 to 6.7, and cultures were routinely grown in a 50°C water bath. This medium supported good growth of the culture without the addition of digestor sludge supernatant, which was originally reported to be required for growth (28).

Washed-cell preparations. Cells were harvested while still in the growth phase, and cells grown on methanol-acetate mixtures were harvested either before they had produced 20 mmol of CH_4 per liter (methanol phase) or after they had produced 25 mmol of CH_4 per liter (acetate phase). The bottles were taken into the anaerobic glove box, the clumps of cells were allowed to settle to the bottom, and ca. 45 ml of the clear supernatant was withdrawn. The remaining cell suspension was washed twice, using a clinical centrifuge inside the glove box, in basal medium to which 20 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) buffer (final pH 6.5) had been added. The washed-cell pellet was resuspended in a small amount of TES-buffered basal medium and dispensed into 9-ml serum vials which were sealed with butyl rubber stoppers. The vials were brought out of the glove box and were flushed with N_2 to remove the H_2 (ca. 1%) that was present in the glove box atmosphere. Anaerobic solutions were then added such that the final reaction mixture had a volume of 2.5 ml and contained approximately 200 μg of cell protein, 20 mM TES buffer (pH 6.5), 0.2 g/liter $Na_2S \cdot 9H_2O$, 2.4 mM $NaHCO_3$, and 10 mM methanol or 10 mM sodium acetate or both (unless otherwise noted). ^{14}C-labeled substrates were added when indicated. The reaction was initiated immediately after these additions were made by placing the vials in a 50°C water bath. These manipulations could be performed in about 1 h, and the washed-cell preparations showed excellent methanogenic activity and usually produced methane without a lag.

Analyses. CH_4 and CO_2 were analyzed by gas chromatography, and $^{14}CH_4$ and $^{14}CO_2$ radioactivity was determined with a gas chromatograph-gas proportional counter system (Packard Instrument Co., Inc., Downers Grove, Ill.) as previously described (27). Protein was extracted from cell samples by boiling them 15 min in 1 M NaOH followed by neutralization with HCl as previously described (27). Protein was analyzed by the Coomassie brilliant blue method with reagents purchased from Bio-Rad Laboratories (Richmond, Calif.). Lysozyme (Sigma Chemical Co., St. Louis, Mo.) was used as a protein standard. In a preliminary report of these results (S. H. Zinder, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, I 88, p. 109), rates of methanogenesis per milligram of protein were erroneously high because bovine serum albumin was used as a protein standard.

Chemicals and radiochemicals. All chemicals were at least reagent grade. [1-^{14}C]sodium acetate (61.6 mCi/mmol), [2-^{14}C]sodium acetate (58.9 mCi/mmol), and $^{14}CH_3OH$ (58 mCi/mmol) were purchased from Amersham Corp. (Arlington Heights, Ill.).

RESULTS

Rates of methanogenesis. Washed cells of acetate-grown *Methanosarcina* sp. strain TM-1 produced methane from acetate at a constant rate during the first few hours of incubation until substrate depletion occurred (Fig. 1). It was from these linear portions of methane production plots that

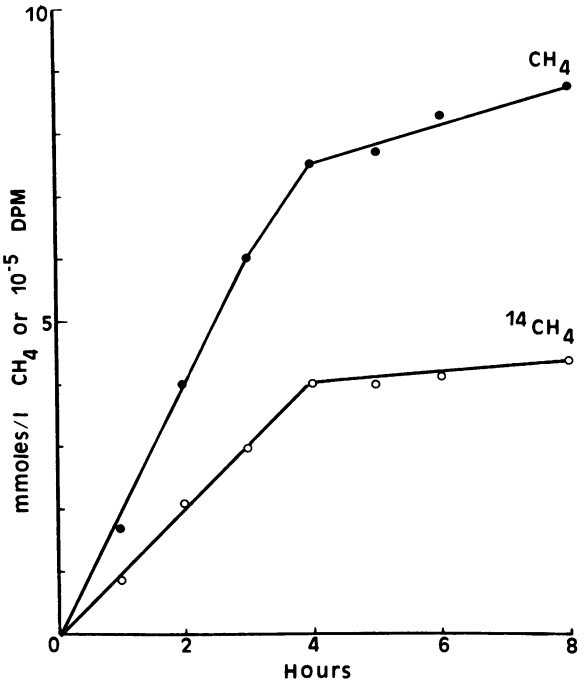

FIG. 1. Production of CH_4 and $^{14}CH_4$ by washed cells of *Methanosarcina* sp. strain TM-1 cells incubated at 50°C with 10 mM sodium acetate and 4.8×10^5 dpm of $^{14}CH_3COO^-$. In this and subsequent figures, "mmol/l CH_4" denotes the amount of methane produced per unit liquid volume rather than a molar concentration.

the rates of methanogenesis in Table 1 were calculated. Production of $^{14}CH_4$ from $^{14}CH_3COO^-$ paralleled CH_4 production, and the average specific activity of the $^{14}CH_4$ produced for all the data points in Fig. 1 was (5.0 ± 0.15 [standard deviation]) $\times 10^4$ dpm/mmol, whereas that for the $^{14}CH_3COO^-$ used was 4.8×10^4 dpm/mmol.

The initial rates of methanogenesis from 10 mM acetate or 10 mM methanol or both by washed cells of *Methanosarcina* sp. strain TM-1 grown on different substrates are presented in Table 1. Acetate-grown cells produced methane from methanol at only about 1% of the rate from acetate. The initial rate of methanogenesis from a methanol-acetate mixture by the acetate-grown cells was nearly identical to that from acetate alone. Methanol-grown cells showed a different pattern of catabolic abilities from that of acetate-grown cells. They produced methane from acetate at a rate ca. 4% of that from methanol. The rate of methanogenesis from a methanol-acetate mixture was nearly double the rate from methanol alone.

TABLE 1. Initial rates of methanogenesis by washed cells of *Methanosarcina* sp. strain TM-1 grown on different substrates and incubated at 50°C with 10 mM acetate or 10 mM methanol or both (unlabeled)

Growth substrate	nmol of CH_4 min^{-1} mg of $protein^{-1}$ with the following substrate added:			
	None	Acetate	Methanol	Acetate-methanol
Acetate	0	325	3	315
Methanol	0	10	280	450
Methanol-acetate (methanol phase)	0	7	220	430
Methanol-acetate (acetate phase)	ND[a]	280	160	435

[a] ND, Not determined.

Cells harvested while still in the methanol phase of growth on a methanol-acetate mixture showed a pattern of methanogenesis nearly identical to that for methanol-grown cells. This was true whether methanol- or acetate-grown cells were used as the inoculum for the methanol-acetate culture (data not presented). Cells harvested in the acetate phase (CH_4 produced, 39 to 44 mmol/liter) were capable of significant rates of methanogenesis from either substrate alone, and the rate of methanogenesis when both substrates were present was nearly equal to the sum of the rates of methanogenesis from each substrate alone.

Methanogenesis by acetate-grown cells. When methanol was present along with acetate in the incubation mixture, the initial rate of methanogenesis by washed acetate-grown cells was identical to the rate from acetate alone (Fig. 2). After about 4 h of incubation, as the acetate was being depleted, additional methanogenesis was detected when methanol was present along with the acetate (Fig. 2). Cells incubated with methanol in the presence of double (20 mM) the usual acetate concentration produced methane at an increased rate after 4 h. The methanol used in this experiment was ^{14}C labeled so that its conversion to methane in the presence of acetate could be examined. In agreement with the results in Fig. 2, little $^{14}CH_4$ was produced from methanol alone by

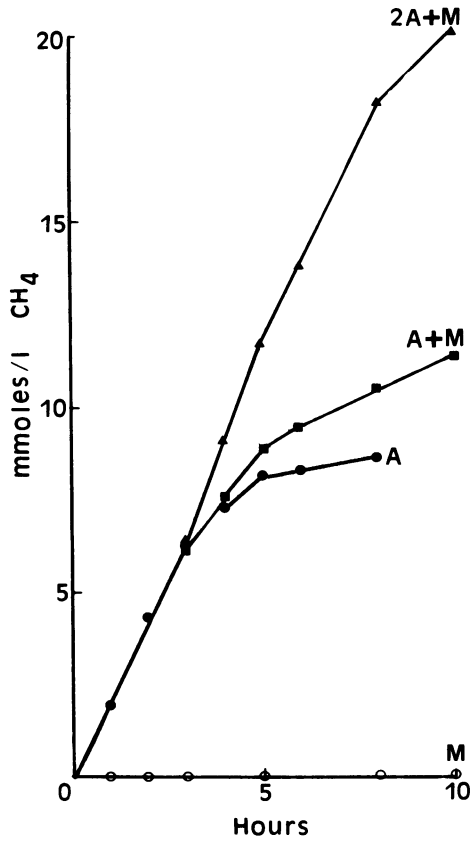

FIG. 2. Methanogenesis from acetate or methanol or both by acetate-grown washed cells of *Methanosarcina* sp. strain TM-1. Abbreviations: M, 10 mM methanol; A, 10 mM acetate; A + M, 10 mM methanol plus 10 mM acetate; 2 A + M, 10 mM methanol plus 20 mM acetate.

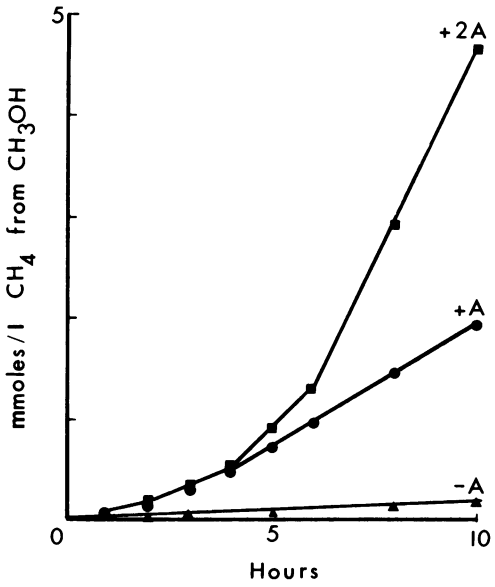

FIG. 3. Effect of acetate on methanogenesis from methanol, calculated by using $^{14}CH_4$ production from $^{14}CH_3OH$, by acetate-grown washed cells of *Methanosarcina* sp. strain TM-1. Abbreviations: −A, no acetate added; +A, 10 mM acetate added; +2A, 20 mM acetate added.

acetate-grown cells (Fig. 3). When 10 mM acetate was present, the rate of methanogenesis from methanol increased over a period of 5 h, after which the rate became linear, coinciding with the time when acetate was becoming depleted in vials with 10 mM acetate (Fig. 2). When 20 mM acetate was present, the rate of methanogenesis from methanol continued to increase throughout the incubation period. Table 2 shows $^{14}CH_4$ and $^{14}CO_2$ production from ^{14}C-labeled substrates by acetate-grown cells. Acetate was split in the usual manner, and methanol, when metabolized along with acetate, showed a $^{14}CH_4/^{14}CO_2$ ratio considerably greater than the theoretical value of 3 for fermentation of methanol alone.

Methanogenesis by methanol-grown cells. Cells grown on methanol in the absence of acetate produced methane more rapidly from a methanol-acetate mixture than the total of the rates of methane produced from methanol and acetate mixtures alone (Fig. 4). Results obtained with ^{14}C-labeled substrates showed that most of this increase was due to an increased rate of methanogenesis from methanol in the mixture. Table 3 shows the products of catabolism of ^{14}C-labeled substrates. The small amount of acetate catabolized when present alone was apparently split in the usual fashion. Methanol was metabolized with a measured $^{14}CH_4/^{14}CO_2$ ratio slightly greater than 3. When methanol was catabolized in the presence of acetate, nearly all of the methanol was reduced to CH_4. In the presence of methanol, methanogenesis from the methyl group of acetate was increased over that for acetate alone (Table 3), and a nearly equal amount of CO_2

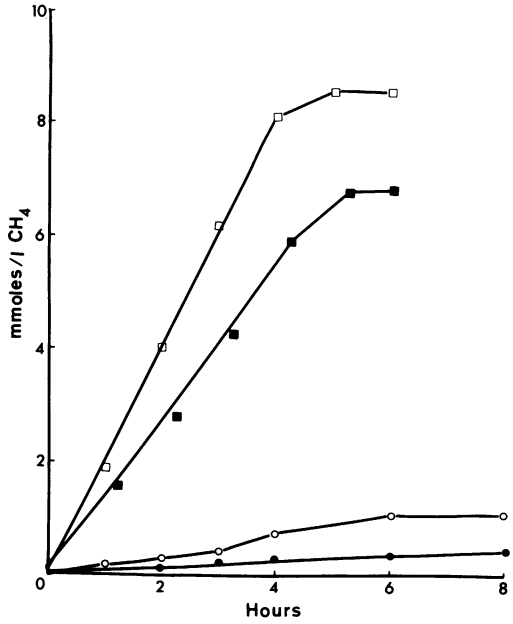

FIG. 4. Methanogenesis, calculated by using $^{14}CH_4$ production from ^{14}C-labeled acetate or methanol, by methanol-grown washed cells of *Methanosarcina* sp. strain TM-1 incubated with acetate, methanol, or acetate-methanol. Symbols: ●, $^{14}CH_3COO^-$ label, substrate was 10 mM acetate; ■, $^{14}CH_3OH$ label, substrate was 10 mM methanol; ○, $^{14}CH_3COO^-$ label, substrate was 10 mM acetate plus 10 mM methanol; □, $^{14}CH_3OH$ label, substrate was 10 mM acetate plus 10 mM methanol.

TABLE 2. Metabolism of ^{14}C-labeled acetate and methanol by acetate-grown cells of *Methanosarcina* sp. strain TM-1[a]

Substrate	Label	CH_4 (mmol/liter)	$^{14}CH_4$ (kdpm[b])	$^{14}CO_2$ (kdpm)	$^{14}CH_4/^{14}CO_2$
Acetate	$^{14}CH_3COO^-$	8.7	432	8	54
Acetate	$CH_3{}^{14}COO^-$	9.3	0	307	0
Methanol	$^{14}CH_3OH$	0.1	11	5	2
Acetate-methanol	$^{14}CH_3COO^-$	10.3	415	11	37
Acetate-methanol	$CH_3{}^{14}COO^-$	11.6	0	307	0
Acetate-methanol	$^{14}CH_3OH$	10.8	120	14	9
2Acetate-methanol	$^{14}CH_3OH$	18.1	238	13	18

[a] Washed cells were prepared as described in Materials and Methods. Results were obtained after 8 h of incubation at 50°C. Substrates were present at 10 mM except in samples denoted 2Acetate in which 20 mM acetate was present. The vials contained 480 kdpm of $^{14}CH_3COO^-$, 530 kdpm of $CH_3{}^{14}COO^-$, or 820 kdpm of $^{14}CH_3OH$, respectively.

[b] kdpm, Thousands of disintegrations per minute per vial.

TABLE 3. Metabolism of ^{14}C-labeled acetate and methanol by methanol-grown cells of *Methanosarcina* sp. strain TM-1[a]

Substrate	Label	CH_4 (mmol/liter)	$^{14}CH_4$ (kdpm)	$^{14}CO_2$ (kdpm)	$^{14}CH_4/^{14}CO_2$
Acetate	$^{14}CH_3COO^-$	0.3	18	1	18
Acetate	$CH_3{}^{14}COO^-$	0.2	0	12	0
Methanol	$^{14}CH_3OH$	6.3	560	162	3.6
Acetate-methanol	$^{14}CH_3COO^-$	9.8	53	45	1.2
Acetate-methanol	$CH_3{}^{14}COO^-$	9.4	0	105	0
Acetate-methanol	$^{14}CH_3OH$	9.3	682	72	9.4

[a] Conditions as described in Table 2, footnote *a*, except that results were obtained after 6 h of incubation.

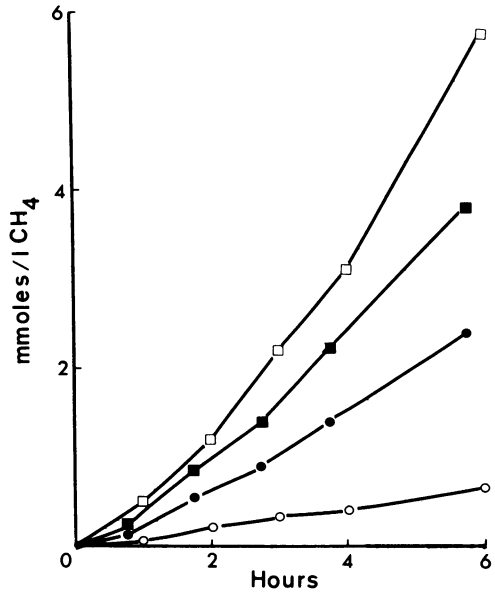

FIG. 5. Methanogenesis, calculated by using $^{14}CH_4$ production from ^{14}C-labeled acetate or methanol, by acetate-phase methanol-acetate-grown cells of *Methanosarcina* sp. strain TM-1 incubated with acetate or methanol or both. Symbols: ●, $^{14}CH_3COO^-$ label, substrate was 10 mM acetate; ■, $^{14}CH_3OH$ label, substrate was 10 mM methanol; ○, CH_3COO^- label, substrate was 10 mM acetate plus 10 mM methanol; □, $^{14}CH_3OH$ label, substrate was 10 mM acetate plus 10 mM methanol.

was produced, indicating a significant amount of acetate oxidation. Although there was slightly greater methanogenesis from acetate in the presence of methanol than in its absence, once methanol was depleted, the rate of methanogenesis from acetate slowed significantly (Fig. 4). $^{14}CO_2$ was the only catabolic product detected from $CH_3{}^{14}COO^-$ incubated in the presence of methanol.

Methanogenesis by cells grown on a methanol-acetate mixture. Cells harvested while still in the methanol phase showed patterns of catabolism nearly identical to those of cells grown on methanol in the absence of acetate (Table 1; data not presented). Cells in the acetate phase were capable of methanogenesis from either acetate or methanol alone (Table 1). To further examine the catabolic capabilities of acetate-phase cells, washed cells which had just entered the acetate phase (CH_4 produced, 27 to 33 mmol/liter) were presented with a mixture of 10 mM each of methanol and acetate, and either $^{14}CH_3OH$ or $^{14}CH_3COO^-$ was added to the vials. The rates of methanogenesis from the labeled substrates when both acetate and methanol were present were compared with the rates when each substrate was present alone (Fig. 5). The rate of methanogenesis for these cells from methanol alone was somewhat greater than that for acetate alone, in accordance with their being early in the acetate phase. When both substrates were present, the rate of methanogenesis from methanol increased while that from acetate decreased. Table 4 shows the distribution of products from ^{14}C-labeled methanogenic substrates by these cells. Acetate was split in the usual fashion when alone, and methanol fermentation resembled that for methanol-grown cells (Table 3). When both substrates were present together, there was significant oxidation of the methyl group of acetate along with greater reduction of methanol to CH_4 but not to the extent found in methanol-grown cells (Table 3).

DISCUSSION

Acetate-grown cells were capable of methanogenesis from methanol at only about 1% of the rate from acetate. This was somewhat surprising since one might expect the metabolism of a preferred substrate to be constitutive. Smith and Mah (19) found that when [^{14}C]methanol (10 mM) was added to cultures of *Methanosarcina barkeri* 227 growing on acetate, significant amounts of $^{14}CH_4$ were produced within an hour of the addition, suggesting that methanol catabolism was constitutive in this strain. Thus, regulatory patterns may be different in different strains of *Methanosarcina* spp.

When methanol alone was added to acetate-grown cells of *Methanosarcina* sp. strain TM-1, there was no increase in the rate of methanogenesis during the 10-h incubation period. However, when acetate was added along with methanol, the methanol was metabolized at increasing rates (Fig. 3) until the acetate was depleted. This increasing rate of methanol utilization with time is reminiscent of an induction curve (5). A possible explanation for an acetate requirement for the shift to methanol by acetate-grown cells is that acetate catabolism provided the energy needed to synthesize inducible proteins responsible for methanogenesis from methanol. These results are similar to those of Gottschal et al. (7) for the aerobic facultative chemolithotroph *Thiobacillus* sp. strain A2, which showed a long lag in the ability to oxidize thiosulfate when acetate-grown cells were presented with thiosulfate, unless some acetate was added along with the thiosulfate. They hypothesized that the acetate was serving as a carbon and energy source for synthesis of proteins involved in thiosulfate oxidation. They also found that cells with high levels of the intracellular reserve material poly-β-hydroxybutyrate were capable of more rapid enzyme induction than cells without it. We have recently found (P. Murray and S. Zinder, manuscript in preparation) that nitrogen-limited, acetate-grown cells of *Methanosarcina* sp. strain TM-1 accumulate an alpha-linked polyglucan and that these cells shift to methanol utilization more rapidly than do carbon-limited cells or cells in balanced growth, which have much lower levels of this polysaccharide.

Methanol-grown cells of *Methanosarcina* sp. strain TM-1 produce methane from acetate alone at a rate about 4% of that from methanol. The rate of methanogenesis from a methanol-acetate mixture by the methanol-grown cells was significantly greater than that from methanol alone. This was mainly due to a stimulation of the rate of methanogenesis

TABLE 4. Metabolism of ^{14}C-labeled acetate and methanol by washed cells of *Methanosarcina* sp. strain TM-1 grown on a methanol-acetate mixture and collected early in the acetate phase[a]

Substrate	Label	CH_4 (mmol/liter)	$^{14}CH_4$ (kdpm)	$^{14}CO_2$ (kdpm)	$^{14}CH_4/^{14}CO_2$
Acetate	$^{14}CH_3COO^-$	2.5	128	5	26
Acetate	$CH_3{}^{14}COO^-$	2.3	0	96	0
Methanol	$^{14}CH_3OH$	3.8	210	59	3.6
Acetate-methanol	$^{14}CH_3COO^-$	7.4	40	25	1.6
Acetate-methanol	$CH_3{}^{14}COO^-$	7.6	0	59	0
Acetate-methanol	$^{14}CH_3OH$	7.5	317	58	5.4

[a] Conditions as described in Table 2, footnote *a*, except that 441 kdpm of $CH_3{}^{14}COO^-$, 538 kdpm of $^{14}CH_3COO^-$, or 554 kdpm of $^{14}CH_3OH$ were added per vial, and incubation was for 6 h.

from methanol by acetate (Fig. 4). This stimulation may be due to the ability of acetate to serve as a carbon source and as a reductant during methanogenesis from methanol (4, 19, 24, 28). These results are consistent with the more rapid growth of *Methanosarcina* sp. strain TM-1 on methanol-acetate mixtures than on methanol alone (28).

Cells grown on a methanol-acetate mixture and harvested before methanol depletion occurred (methanol phase) had rates of methanogenesis from acetate, methanol, and methanol-acetate which were nearly identical to those for cells grown on methanol alone (Table 1). Specifically, the presence of 40 mM acetate in the growth medium did not significantly increase the ability of the cells to use acetate alone for methanogenesis.

Cells harvested after methanol depletion (acetate phase) were capable of methanogenesis at significant rates from either methanol or acetate alone, demonstrating that these activities are not necessarily mutually exclusive. The ability of cells to use methanol was apparently residual from growth during the methanol phase. From growth yield data for *Methanosarcina* sp. strain TM-1 (28), it can be calculated that the cells go through less than one mass doubling after shifting to acetate in growth medium containing 24 mM methanol and 40 mM acetate. Acetate-phase cells, when presented with both 10 mM acetate and 10 mM methanol, showed a stimulation in the rate of methanogenesis from methanol and a decrease in the rate from acetate, indicating a kinetic preference for methanol under these conditions.

These results are consistent with a model for regulation of catabolism in *Methanosarcina* sp. strain TM-1 in which methanol represses methanogenesis from acetate, while methanogenesis from methanol is itself inducible. Evidence for methanol repression of acetate catabolism is that cells grown on methanol in the presence of 40 mM acetate showed the same low rate of methanogenesis from acetate as did cells grown on methanol with no acetate added. This cannot be explained by a simple kinetic preference for methanol over acetate or by reversible inhibition of acetate-specific enzymes by methanol. Genetic events such as a mutation, phase variation, or transposon movement are unlikely explanations for the differences seen in the cells grown on different substrates, because the changes occurred too rapidly to be caused by such rare genetic events. For example, when acetate-grown cells were used as an inoculum for growth in methanol-acetate medium, cells harvested in the methanol phase, representing only a few generations of growth in this medium, showed as little ability to use acetate as did a methanol-grown inoculum. These results do not rule out the possibility that genetic events are involved in long lag periods found in other strains of *Methanosarcina* spp.

That methanogenesis from methanol is inducible is supported by the inability of acetate-grown cells to use methanol, yet addition of methanol caused a rapid increase in the ability to use methanol in the presence of acetate. Clearly, acetate did not repress methanogenesis from methanol and was actually required, apparently as an energy substrate, for a shift to methanol catabolism by acetate-grown cells. A similar requirement for a cosubstrate during metabolic shifts may partially explain the long lags sometimes encountered when methanol-grown cells of some strains of *Methanosarcina* spp. are transferred to acetate medium. If the preferred substrate strongly represses acetate catabolism in these strains and if the cells do not contain significant amounts of an endogenous energy reserve, they will not have the energy available to synthesize proteins needed for acetate catabolism.

To prove that the proposed induction and repression are actually occurring, it must be shown that synthesis of polypeptides specific to methanogenesis from acetate is repressed by methanol and that synthesis of polypeptides specific to methanogenesis from methanol requires methanol. Possible methanol-specific polypeptides would include the methanol-methylcoenzyme M transferases described by Van der Meijden et al. (23), enzymes involved in biosynthesis of the methyl group of acetyl coenzyme A from methanol (10), and proteins involved in methanol transport, if it is transported. In terms of acetate-specific activities, it has been shown that carbon monoxide dehydrogenase activity is fivefold greater in acetate-grown than in methanol-grown cells of *Methanosarcina barkeri* MS (12), although an increase in a specific polypeptide has not yet been demonstrated. This enzyme, which may play an important role in CO_2 formation from the carboxyl group of acetate (12), is also apparently involved in the biosynthesis of the carbonyl group of acetyl coenzyme A in methanol-grown cells (10), so it is not surprising that significant activity is found in methanol-grown cells. Other acetate-specific activities could include acetate thiokinase (11) and acetate transport proteins, if acetate is transported.

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