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Acetate as Sole Carbon and Energy Source for Growth of *Methanosarcina* Strain 227

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Methanosarcina strain 227 grew rapidly and produced methane on a mineral medium containing acetate as the sole added organic substrate. Cell yields but not doubling times were affected by the presence or absence of yeast extract. Greater cell yields occurred in yeast extract medium than in mineral medium. Radioactive labeling studies showed that acetate was decarboxylated in mineral medium, as was shown previously in complex medium. The specific radioactivity of methane produced per specific acitvity of acetate added was not significantly different in yeast extract medium compared with mineral medium. Unequivocal evidence indicates that the cleavage of acetate to methane and carbon dioxide provided the energy for growth in the presence or absence of other organic compounds; these latter compounds do not serve as energy sources, electron donors, or significant sources of methane during this aceticlastic reaction.

Acetate was first reported as a substrate for methanogenesis in 1876 when Hoppe-Seyler added acetate to sewage sludge (8). He later showed that enrichment cultures metabolized acetate to form equimolar quantities of methane and carbon dioxide (9). Subsequent experiments by Buswell and Sollo (5), Stadtman and Barker (20, 21), and Pine and Barker (16) demonstrated that acetate was decarboxylated by impure cultures. The methyl group of acetate, together with its hydrogen atoms, was converted to methane, and the carboxyl group was converted to carbon dioxide.

Early experiments by Soehngen, in which he described acetate-using methanogenic bacteria, were performed with impure cultures (N. L. Soehngen, Ph.D. thesis, Technical University of Delft, Rotterdam, The Netherlands, 1906). In 1947, Schnellen isolated and cultured *Methanosarcina barkeri* on methanol or H_2-CO_2 as substrate and showed that it grew slowly on acetate (C. Schnellen, Ph.D. thesis, Technical University of Delft, Rotterdam, The Netherlands, 1947). However, acetate splitting was not demonstrated and the original cultures of Schnellen were lost. Later isolates of *Methanosarcina* did not produce methane from acetate at ecologically significant rates (26).

Objections against the decarboxylation of acetate as a means of producing energy for adenosine 5'-triphosphate synthesis and growth were raised because of thermodynamic and mechanistic considerations (2, 17, 26, 27). To answer these objections, the conversion of acetate to methane and carbon dioxide was hypothesized to occur by one of the following routes: (i) complete oxidation of each 1 mol of acetate to 2 mol of CO_2 and 8H (the latter would be used subsequently to reduce 1 mol of CO_2 to CH_4 for every 1 mol of acetate oxidized) (2); (ii) complete reduction of 1 mol of acetate to 2 mol of methane by hydrogen or reductants generated from the oxidation of organic compounds other than acetate (reduction of acetate by this mechanism would produce sufficient energy for adenosine 5'-triphosphate synthesis and growth) (27, 29); or (iii) decarboxylation of 1 mol of acetate to 1 mol of methane and 1 mol of carbon dioxide by cometabolism with an energy source other than acetate (17, 29).

Hypotheses i and ii were not consistent with the data of Buswell and Sollo (5), Stadtman and Barker (20, 21), and Pine and Barker (16). All three hypotheses were invalidated by pure culture work on *Methanosarcina*, which showed that growth on acetate was dependent on the quantity of acetate converted to methane and that radioactive acetate was split by a decarboxylation (12, 19). Cometabolism of another compound was not involved. Nevertheless, the notion that supplementary energy sources are required for growth and/or methanogenesis from acetate has persisted (23).

In this paper we show that *Methanosarcina* strain 227 can grow on acetate as the sole source of organic carbon and energy. The data presented here rule out cometabolism, acetate reduction, and acetate oxidation to CO_2 followed by CO_2 reduction to methane as mechanisms for growth on acetate. In addition, experiments on the stimulatory role of yeast extract (YE) during growth on acetate are presented.

MATERIALS AND METHODS

Bacterial culture. *Methanosarcina* strain 227 was isolated as described previously (12).

Culture media. All solutions were prepared and handled under oxygen-free 100% N_2. The basal salts solution for the mineral acetate medium contained 1.0 g of NH_4Cl per liter, 0.4 g of $K_2HPO_4 \cdot 3H_2O$ per liter, 10.7 g of $Na_2HPO_4 \cdot 7H_2O$ per liter, 6.8 g of $NaC_2H_3O_2 \cdot 3H_2O$ per liter, and 0.0001% resazurin. After the medium was boiled under N_2, its pH was adjusted to 6.5 to 6.7 with 12 N HCl before it was dispensed in 50-ml portions into 125-ml serum vials and autoclaved. After autoclaving, 0.5 ml of a sterile aqueous solution containing $MgCl_2 \cdot 6H_2O$ (1.0 g/100 ml) and $CaCl_2 \cdot 2H_2O$ (1.0 g/100 ml) was added to each vial. The medium was reduced with 0.5 ml of a sterile, anaerobic solution containing 1% $Na_2S \cdot 9H_2O$ before inoculation, giving a final concentration of 0.01% Na_2S. No cysteine was used in this medium.

Any additional supplements to the mineral acetate medium were also prepared as sterile, anaerobic concentrated solutions under 100% N_2 and were added to the medium before reduction with the Na_2S solution. YE ash was prepared by combusting YE in a muffle furnace at 650°C (1). The YE ash was dissolved in sufficient distilled water to give a solution equivalent to 10% YE. This solution was boiled under 100% N_2 and sterilized by autoclaving.

YE-Trypticase medium containing the following (in grams per liter): NH_4Cl, 1.0; $K_2HPO_4 \cdot 3H_2O$, 0.4; $MgCl_2 \cdot 6H_2O$, 0.1; YE (BBL Microbiology Systems, Cockeysville, Md.), 2.0; Trypticase (BBL Microbiology Systems), 2.0; $NaC_2H_3O_2 \cdot 3H_2O$, 6.8; and cysteine-hydrochloride, 0.5. Resazurin and $Na_2S \cdot 9H_2O$ were prepared and added to final concentrations of 0.0001 and 0.01%, respectively, as previously described. The pH before autoclaving was adjusted to 6.7 with 12 N HCl. The gas atmosphere was 100% N_2.

Cultures were maintained on a mineral acetate medium for a minimum of three transfers by using a 1% inoculum before inoculation into experimental media. Strain 227 has been carried in this medium over numerous transfers for about 1 year. Culture volumes and growth conditions were as described previously (29).

Growth yields. Growth yields were determined as described previously (19).

Methane determinations. Methane and carbon dioxide were determined on a Varian Aerograph gas chromatograph as described previously (15, 19).

Isotopes. Sodium [2-^{14}C]acetate (specific activity, 48 to 58 mCi/mmol) was purchased from International Chemical and Nuclear Corp., Irvine, Calif., or Schwarz/Mann, Orangeburg, N.Y. Sodium [1-^{14}C]acetate (specific activity, 57 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill. Sodium L-[*U*-^{14}C]acetate (specific activity, 98 mCi/mmol) was purchased from Schwarz/Mann.

Labeling studies. Experiments with radioactive substrates were performed in 125-ml serum vials containing 50 ml of medium and 5 to 10 μCi of ^{14}C-labeled substrate. A 1 to 3% inoculum of *Methanosarcina* strain 227 grown in mineral acetate medium was used for all experiments. In experiments in which the total quantity of CO_2 produced was determined, the cultures were acidified with 1 ml of 4 N HCl and allowed to stand at room temperature for 1 to 2 h before the headspace gases were measured. Such cultures were shaken vigorously several times during this period to allow equilibration of the CO_2 in the headspace. Samples were again monitored after standing for 24 h. Significant differences in CO_2 were not noted between samples taken 24 h after acidification and those taken 1 h after acidification. Total CO_2 was calculated from the measured CO_2 in the headspace, and the dissolved CO_2 in the culture medium was estimated by using Henry's Law.

CH_4, CO_2, $^{14}CH_4$, and $^{14}CO_2$ were determined as described previously (1, 15). Samples of culture liquid (0.5 to 1.0 ml) or filtered cells were counted in a Beckman liquid scintillation counter in scintillation vials containing 10 ml of Aquasol scintillation fluid (New England Nuclear Corp., Boston, Mass.). Counts per minute were corrected to disintegrations per minute.

RESULTS

Methanogenesis on mineral acetate medium. Strain 227 was transferred on mineral acetate medium in our laboratory for more than 25 transfers by using small (1 to 2%) inocula. Initial transfers from 0.2% YE–Trypticase medium into mineral acetate medium resulted in rapid growth and final cell densities nearly as high as the density obtained in YE–Trypticase medium. However, after about eight successive transfers into mineral acetate medium, the final cell densities were much less than the density on YE–Trypticase medium. Similar findings were also observed when methanol was the primary substrate. Large inocula (about 6%) were needed for consistent growth on mineral acetate medium, even with YE ash in the medium. A requirement for large inocula on mineral acetate medium was also reported by Zhilina (28).

We recently demonstrated growth and methanogenesis of a pure culture of *Methanosarcina* strain 227 by decarboxylation of sodium acetate in complex (YE) medium in the absence of added H_2 (12, 19). The role of YE in this complex medium was further examined in the following experiments to evaluate the unlikely hypothesis that YE supplied reducing equivalents or methanogenic substrates, as suggested by others (23).

Duplicate 125-ml serum vials containing 50 ml of mineral acetate medium or 50 ml of mineral acetate medium supplemented with 0.1% YE ash, 0.1% YE, or 0.5% YE were inoculated with 1 ml of a culture of *Methanosarcina* strain 227 which was transferred six times on unsupplemented mineral acetate medium. Methanogenesis was monitored as previously described.

Figure 1 shows that methanogenesis on mineral acetate medium in a nitrogen atmosphere

was exponential and that growth therefore occurred on this medium. Addition of YE or YE ash increased the period of exponential methanogenesis but did not appear to affect the doubling time significantly (Table 1). YE seemed to be more stimulatory than YE ash, and 0.5% YE resulted in slightly more methane than 0.1% YE. This behavior toward YE was previously reported for *Methanosarcina* strain 227 during growth on methanol and in acetate enrichments and suggests that YE owes its effect principally to its mineral content and to the higher cell densities achieved rather than to a faster doubling time (1, 11, 12). Growth and methanogenesis on mineral acetate medium unequivocally demonstrated that energy for growth must be obtained during methanogenesis from acetate.

The manner in which acetate was dissimilated to CH_4 and CO_2 by strain 227 in a mineral acetate medium was determined by radioactive labeling. Strain 227 was transferred to duplicate vessels containing mineral acetate medium with and without YE ash equivalent to 0.1% YE. Sodium [1-^{14}C]acetate (5 μCi) or sodium [2-^{14}C]acetate (5 μCi) was added to the medium before inoculation. CH_4, CO_2, and the radioactive gases in the headspace were monitored as described above. Figure 2 shows that the methyl group of acetate was converted to methane and the carboxyl group was converted to CO_2. This agrees with results from our earlier experiments with cells growing on acetate in a complex medium. Mechanisms of acetate catabolism that involve reduction of CO_2 or acetate by reducing equivalents generated from oxidation of acetate to CO_2 as an intermediate step in methanogenesis are thus ruled out as a major route of methanogenesis from acetate.

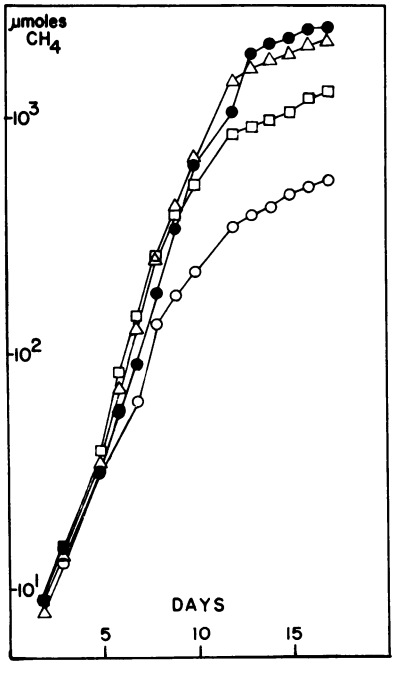

FIG. 1. *Methanogenesis from acetate in mineral medium and supplemented mineral media. Symbols: Quantity (micromoles) of CH_4 in unsupplemented mineral medium (○), mineral medium containing 0.1% YE ash (□), mineral medium containing 0.1% YE (△), and mineral medium containing 0.5% YE (●).*

TABLE 1. *Growth on mineral and complex media*[a]

Medium	μ_{CH_4} (days^{-1})	Y_{CH_4} (mg of cells/mmol of CH_4)	q_{CH_4} (mmol of CH_4/mg of cells per day)[b]
MA[c]	0.441	3.17	0.139
MA + 0.1 YE ash	0.514	2.53	0.211
MA + 0.2% YE ash	0.277	2.21	0.126
MA + 0.1% YE	0.555	3.41	0.163
MA + 0.2% YE	0.407	2.89	0.141
MA + 0.5% YE	0.482	2.81	0.179
YE-trypticase	0.385	3.08	0.125

[a] The growth substrate was 50 mM acetate.
[b] $q_{CH_4} = \mu_{CH_4}/Y_{CH_4}$.
[c] MA, Mineral acetate basal medium.

Effect of YE medium on growth. The effect of YE on *Methanosarcina* was examined by inoculating cultures into mineral acetate medium or mineral acetate medium supplemented with YE or YE ash and measuring the dry weight of cells and methanogenesis as indices of growth. Determinations of the specific methanogenic rate constant (which is the same as the specific growth rate constant [μ_{CH_4}]) (19, 29), the molar growth yield (Y_{CH_4}), and the specific rate of methanogenesis (q_{CH_4}) were made. We define q_{CH_4} by the following formula: $q_{CH_4} = \mu_{CH_4}/Y_{CH_4}$, where q_{CH_4} is the rate of methane production (in millimoles of CH_4 per day) per unit cell mass (dry weight). The results of these experiments are shown in Tables 1 and 2.

In Table 1, μ_{CH_4}, Y_{CH_4}, and q_{CH_4} are compared for cultures in mineral acetate medium and mineral acetate medium supplemented with YE ash or various concentrations of YE. Significant differences in q_{CH_4} and μ_{CH_4} were not observed. Although slightly higher average values of Y_{CH_4} were found for media with YE than for media without YE, the differences may not be significant. The results suggested that YE does not greatly affect the growth rates or growth efficiency of cultures on acetate, although final cell yields may be affected.

Table 2 shows the effect on growth of varying the acetate concentrations or of substituting

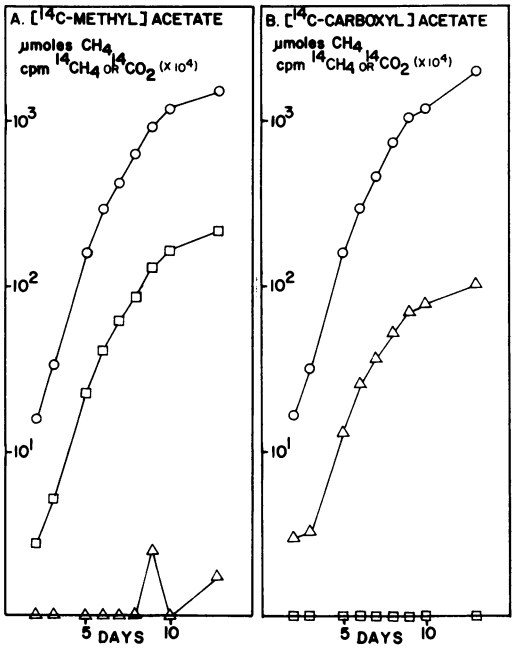

FIG. 2. *Radioactive gases produced from radioactive acetate on mineral medium. Symbols: Quantity (micromoles) of CH_4 (○), $^{14}CH_4$ (□), and $^{14}CO_2$ (△).*

TABLE 2. *Effect of acetate and methanol on methanogenesis*[a]

Growth substrate, mM	μ_{CH_4} (days^{-1})	Y_{CH_4} (mg of cells/mmol of CH_4)	q_{CH_4} mmol of CH_4/mg of cells per day)
Acetate, 10	0.241	1.72	0.164
Acetate, 30	0.411	2.12	0.198
Acetate, 50	0.473	1.92	0.128
Acetate, 100	0.421	1.96	0.218
Methanol, 100	0.821	5.10	0.163

[a] The basal medium was YE-Trypticase medium and was supplemented with the indicated growth substrate.

methanol for acetate. Faster growth rates (higher values of μ_{CH_4}) were obtained at the higher acetate concentrations than at the lower acetate concentrations. However, Y_{CH_4} and q_{CH_4} appeared to be unaffected by the changes in the acetate concentration. This suggests that growth efficiencies were similar at the higher and lower acetate concentrations. Similar values of q_{CH_4} were obtained regardless of whether the growth substrate was acetate or methanol.

Fate of radioactive acetate during growth on mineral and complex media. The extent of acetate conversion into cells and gases was examined by inoculating a mineral acetate medium-grown culture of strain 227 into mineral acetate medium or supplemented mineral acetate medium containing radioactive acetate. After incubation at 35°C for 20 days, samples were assayed for radioactivity in cells and filtrates. The cultures were then each acidified with 1 ml of 4 N HCl and equilibrated for 2 h. The headspace gases were then assayed for CH_4 and CO_2. The calculated values for the gases accounted for the solubility of CO_2 in the culture liquid (Tables 3 and 4).

Approximately 80 to 100% of the radioactivity added as acetate to the cultures was accounted for in the cells, headspace gases, and culture filtrates (Table 3). About 2 to 3% of the [2-^{14}C]acetate converted to methane was incorporated into cells (Table 4). Somewhat more radioactivity was incorporated into cells grown on media supplemented with YE than into cells grown with YE ash or on unsupplemented mineral acetate medium. A larger fraction of the acetate metabolized to methane was incorporated into cells for carboxyl-labeled or uniformly labeled acetate than for methyl-labeled acetate. This preferential incorporation of carboxyl-labeled acetate may reflect CO_2 fixation into cells since acetate was the only source of CO_2 in the medium.

The distribution of radioactivity into gases is also of interest in view of the report based on indirect evidence suggesting that significant quantities of methane are produced from YE during growth of *Methanosarcina barkeri* in complex media (23). Table 4 shows that nearly all of the radioactivity from methyl-labeled acetate appeared in methane. About 1 to 3% of [2-^{14}C]acetate was oxidized to $^{14}CO_2$. Conversely, nearly all the [1-^{14}C]acetate metabolized was converted to $^{14}CO_2$. Only 0.2 to 0.5% appeared as $^{14}CH_4$. In previous experiments, traces of $^{14}CH_4$ were not observed from [1-^{14}C]acetate. The reason for the small quantities produced in these experiments is not clear. Uniformly labeled acetate was converted to approximately equal quantities of $^{14}CH_4$ and $^{14}CO_2$. The radioactive labeling studies showed that acetate was cleaved to form CH_4 and CO_2 in the presence or absence of YE. Computations of the relative specific activities of the gases and added acetate (allowing also for the contribution of acetate by YE and Trypticase [2 μmol/ml]) support this. Moreover, duced compared with that of the added acetate was independent of the presence of YE in the medium. We therefore conclude that methane is not formed in any significant quantity from YE during growth on acetate. We also conclude that acetate may serve as sole source of carbon and energy for growth.

DISCUSSION

Methanosarcina strain 227 was maintained for over 1 year (about 25 transfers) on mineral

TABLE 3. *Distribution of radioactivity after growth on [^{14}C]acetate in mineral or supplemented medium*

Isotope	Medium	Radioactivity (× 10^6 dpm) in:				% Recovery
		Acetate added	Culture supernatant	Cells	Headspace	
[2-^{14}C]acetate	MA[a]	1.746	0.872	0.011	0.769	94.6
	MA	1.238	0.966	0.007	0.323	104.6
	MA + 0.1% YE ash	1.658	1.149	0.008	0.494	99.6
	MA + 0.1% YE	1.491	0.152	0.036	1.468	111.1
	MA + 0.1% YE	1.406	0.143	0.033	1.659	130.5
[1-^{14}C]acetate	MA	11.681	8.790	0.115	0.594	81.3
	MA	13.096	9.265	0.160	0.876	78.7
L-[*U*-^{14}C]acetate	MA	15.091	7.522	0.188	3.265	72.7[b]
	MA	15.260	8.406	0.156	2.774	74.2[b]
	MA	14.692	10.389	0.142	2.306	87.4
	MA	19.192	13.448	0.179	4.019	91.9

[a] MA, Mineral acetate medium.

[b] The culture supernatant liquid for the two cultures on mineral acetate medium was accidentally diluted by an unknown amount, which resulted in the low recoveries.

TABLE 4. *Distribution of radioactivity into gases and cells after growth on [^{14}C]acetate in mineral or supplemented medium*

Isotope	Medium	Amt (μmol) of:		Radioactivity (× 10^6 dpm) in:		Sp act ratio[b]	Cells/CH_4[c]
		CH_4	CO_2[a]	CH_4	CO_2[a]		
[2-^{14}C]acetate	MA	903	1,052	0.542	0.026	0.86	0.020
	MA	564	603	0.274	0.006	0.98	0.026
	MA + 0.1% YE ash	677	792	0.402	0	0.89	0.020
	MA + 0.1% YE	2,152	2,243	1.042	0.030	0.81	0.035
	MA + 0.1% YE	2,464	2,412	1.064	0.045	0.89	0.031
	MA + 0.5% YE	640	701	0.353	0	0.94	0.014
[1-^{14}C]acetate	MA	752	841	0.004	2.248	0.86	0.051
	MA	707	799	0.015	3.400	0.81	0.047
	MA + 0.5% YE	2,904	3,162	0.010	8.794	0.65	0.084
	MA + 0.5% YE	1,218	1,245	0.016	4.886	0.86	0.066
L-[*U*-^{14}C]acetate	MA	752	841	1.954	2.125	0.43	0.096
	MA	654	743	1.764	1.842	0.44	0.088
	MA + 0.1% YE ash	564	631	1.605	1.653	0.48	0.089
	MA + 0.1% YE ash	655	729	2.416	2.361	0.48	0.074

[a] Total CO_2 was estimated from the quantity of gas in the headspace over the culture and from the calculated solubility of CO_2 in the medium.

[b] Specific activity of CH_4/specific activity of acetate. The specific activity of acetate was calculated from the estimated quantity of acetate added and the measured radioactivity of the culture before inoculation. The specific activity ratio is given as specific activity of CO_2/specific activity of acetate for [1-^{14}C]acetate.

[c] Disintegrations per minute in cells/disintegrations per minute in CH_4, except when [1-^{14}C]acetate was used; for [1-^{14}C]acetate, disintegrations per minute in cells/disintegrations per minute in CO_2.

acetate medium composed of reagent-grade chemicals. Strain 227 and other strains of *Methanosarcina* (strains MS, UBS, biotype 2, and strain W) were carried previously on a mineral acetate medium different in composition from the one described here and composed of technical-grade chemicals (L. Baresi, Dr. P.H. thesis, University of California at Los Angeles, Los Angeles, 1978; T. J. Ferguson, unpublished data).

It has been our experience that cultures on mineral acetate or mineral methanol medium are more difficult to establish and maintain than cultures in complex medium. Initial lack of reproducible growth made interpretation of experiments comparing growth in defined medium versus growth in YE medium difficult (12). The medium used in the experiments reported here provided greater reproducibility than the defined medium reported previously. Although growth and methanogenesis occurred with a 1% inoculum of strain 227 on mineral acetate medium, best results were obtained with inocula of 5 to 6%. Difficulty in establishing cultures on mineral acetate medium may be related to the lack of poising capacity and maintenance of a

low oxidation-reduction potential, which organic compounds in a complex medium may provide.

The results reported here show that acetate can serve as the sole source of carbon and energy. Cell mass increased during exponential production of methane from acetate in a mineral salts medium devoid of any other organic compound. Addition of YE to the mineral medium resulted in greater cell and final methane yields. Neither μ_{CH_4} nor q_{CH_4} was significantly affected by the presence or absence of YE in the mineral medium. A slight increase in Y_{CH_4} was observed in cultures with added YE, compared with similar cultures in mineral medium. These observations show that YE may stimulate growth on acetate but that it is not required for growth. Similar results have been obtained with *Methanosarcina* grown on methanol (12).

The nature of the stimulation by YE is not clear. Its lack of significant effect on q_{CH_4} and μ_{CH_4} suggests that the mode of growth may not be fundamentally different on complex medium compared with mineral medium. The slight increase in Y_{CH_4} on YE medium compared with mineral medium may not be significant or may reflect utilization of organic constituents of YE for biosynthetic purposes, suggesting that the mineral acetate medium may be nutritionally limiting.

The radioactive isotope labeling data show that acetate is decarboxylated during growth in mineral or complex medium. Small quantities of $^{14}CO_2$ (1 to 4% of the radioactivity appearing in methane) were produced from $^{14}CH_3COOH$ in both mineral and YE media. This suggests that acetate is oxidized to generate reducing equivalents for biosynthetic reactions in the presence or absence of YE and suggests that fundamental differences in the catabolism of acetate do not occur as a result of adding YE to the medium. Radioactive methane was also produced from $CH_3{}^{14}COOH$ in small quantities in both mineral and YE media. These small quantities of radioactive methane could also result from trace contamination by methyl-labeled acetate during commercial isotope preparation or by reduction of radioactive CO_2 with reducing equivalents generated during cell metabolism. Winter and Wolfe (24) reported large amounts (10% of methane or CO_2 formed) of $^{14}CH_4$ from carboxyl-labeled acetate and $^{14}CO_2$ from methyl-labeled acetate. The differences from our results can probably be explained by the differences in the methods used for determining radioactive methane and carbon dioxide.

Acetate and carbon dioxide were the sole carbon sources available in our mineral medium. Labeling studies showed that *Methanosarcina* incorporated about 2 to 3 mol of methyl-labeled acetate into cell material for each 100 mol converted to methane. The amount of acetate assimilated was similar in both media, suggesting that acetate and carbon dioxide were the primary sources of cell carbon even in the presence of YE. Preferential incorporation of carboxyl-labeled and uniformly labeled acetate instead of methyl-labeled acetate suggested that significant quantities of CO_2 are fixed during growth on acetate, which is in agreement with previous findings of Zinder and Mah (29). Weimer and Zeikus (22, 23) reported that up to 60% of the cell carbon formed during growth on acetate in complex medium was derived from acetate and that 50% of the cell carbon was derived from CO_2 during growth on methanol.

M. barkeri is the most metabolically versatile methanogenic species in pure culture. It is capable of growth and/or methane production with H_2–CO_2, methanol, acetate, methylamines, or carbon monoxide as substrate (3, 7, 10, 13, 25, 26). In preliminary experiments performed in our laboratory, some ^{14}C-labeled amino acids (other than cysteine) were taken up and incorporated by *Methanosarcina* into cell material during growth on acetate in YE medium (unpublished data). Trace quantities of radioactivity were detected in methane and carbon dioxide. These findings suggested that some organic components of YE (such as amino acids) may be used for biosynthetic purposes. Amino acids were, in fact, reported to serve as growth requirements for *Methanobacterium ruminantium* strain M1 (4).

A comparison of the specific radioactivities of CH_4 and CO_2 produced from radioactive acetate in YE medium compared with mineral medium showed that YE had no effect on the specific activities of the headspace gases. The distribution of label in CH_4 and CO_2 was also unaffected by YE in the medium. These findings, together with our previous results, suggested that methanogenesis and growth occurred primarily by the same metabolic pathways in mineral medium as they did in YE medium (12, 19). Moreover, acetate served as the sole source of energy in both media.

The mechanistic and thermodynamic problems raised by growth on acetate using a decarboxylation reaction remain unsolved. Recent evidence suggests a role for chemiosmotic mechanisms of energy generation in methanogenic bacteria (6, 14, 18).

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