STUDIES ON THE METHANE FERMENTATION

X. A New Formate-Decomposing Bacterium, Methanococcus vannielii[1]

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The methane fermentation of formate was discovered by Söhngen (1906) as a result of the addition of the calcium salt to an enrichment culture of butyrate-fermenting bacteria. Söhngen found that formate was decomposed more rapidly than butyrate or acetate, and was converted almost quantitatively to methane and carbon dioxide. The responsible bacteria were not isolated or described. Many years later, Coolhaas (1928) observed a methane fermentation of formate, apparently caused by a rod-shaped thermophilic bacterium which also was not isolated. In this fermentation, a little hydrogen was formed along with methane and carbon dioxide. More recently, several pure or purified cultures of methane-producing bacteria were shown to decompose formate. Stephenson and Stickland (1933) claimed to have isolated a pure culture of a bacterium that grew on media containing formate as a sole energy source and also metabolized other one-carbon compounds, but was unable to use multicarbon compounds such as ethanol and acetate. However, the organism was not named or described, and no conclusive evidence was presented to establish the purity of the culture. The first undoubtedly pure culture of a highly specialized formate-decomposing methane bacterium was isolated by Schnellen (1947) and given the name *Methanobacterium formicicum*. This rod-shaped bacterium requires formate as an energy source and is unable to use other compounds except hydrogen, carbon monoxide, and carbon dioxide (Kluyver and Schnellen, 1947). Barker (1941) found that *Methanobacterium omelianskii* also is able to decompose formate under special conditions, but it cannot use formate as a growth substrate.

In the present paper, we shall describe the isolation and characteristics of a new type of formate-decomposing methane bacterium, *Methanococcus vannielii*, and present the results of a few biochemical experiments with this organism.

METHODS

Growth was determined turbidimetrically, using an Evelyn photoelectric colorimeter with a 660 mμ filter. Manometric studies with cell suspensions were conducted in the conventional Warburg apparatus at 37 C.

Formate was recovered for analysis by steam distillation in the apparatus described by Markham (1942) and estimated by titration or by oxidation to CO_2 with HgO according to the method of Friedemann (1938). Carbon dioxide and methane were recovered and estimated as described previously (Stadtman and

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Barker, 1949). Carbon dioxide and formate were converted to $BaCO_3$ for radioactivity measurements.

EXPERIMENTAL RESULTS

Enrichment cultures in formate media. Enrichment for an organism capable of causing a methane fermentation of formate was undertaken by inoculating a mineral-bicarbonate medium (Stadtman and Barker, 1951) containing 1 per cent sodium formate with black mud from the east shore of San Francisco Bay. The primary cultures required about 2 weeks' incubation at 30 C before gas production became rapid, but in successive transfers, using a 10 per cent inoculum, a vigorous fermentation was obtained in 16 to 36 hours, and the medium became alkaline (pH 8 to 9).

Since methane bacteria generally develop rather slowly, it seemed unlikely that the abundant gas evolved in these rapid fermentations was methane. However, an analysis of 50 ml of gas obtained from a 250 ml culture during the first 18 hours of fermentation showed it to be mainly methane with a little carbon dioxide. No hydrogen was detected.

Examination of the formate enrichment cultures showed that the most conspicuous organism was a motile coccus. Since none of the previously described methane bacteria was of this morphological type, the isolation of the organism was undertaken. It was hoped that its rapid growth rate and distinctive appearance would facilitate purification.

Isolation of a pure culture. A series of tubes containing a 1 per cent formate-agar medium, pH 7.2, were inoculated with a small amount of a vigorously fermenting enrichment culture according to the method of Barker (1936). Colonies developed rapidly in the various dilutions, and the agar became alkaline and was split by gas. In the sixth dilution, a single, 1 to 2 mm colony developed which produced visible gas after 6 days. This colony contained two types of bacteria, the motile coccus and a highly motile straight rod. Agar shake tubes inoculated with this colony also produced gas, but in this new series, the coccus grew only in the lower dilutions and the colonies were very small. A few colonies of the rod were found in the higher dilutions. Numerous other similar series of shake tubes were made and always gave the same results. Addition of 0.1 per cent yeast extract (Difco) stimulated growth of the rod-shaped bacterium, causing the medium to become very alkaline and the agar to be split by gas. However, the coccus still was found only in the lower dilutions. This suggested that the rod rather than the coccus might be the methane-producing organism.

A pure culture of the rod-shaped bacterium was readily obtained in the formate-agar medium supplemented with 0.1 per cent yeast extract. This culture was tested for the ability to form methane by culturing it in 100 ml of a mineral medium containing 1 per cent sodium formate, 0.1 per cent yeast extract, and 0.2 per cent Na_2CO_3. The fermentation appeared to be complete after a 4 day incubation period at 30 C. Although the medium was alkaline (pH 8.0), only 15 ml of gas had been collected. Analysis of the gas showed that no methane had been produced. Instead, carbon dioxide (0.06 mM) and hydrogen (0.35 mM)

were found. It was concluded that the coccus had been responsible for the methane formation in the earlier cultures.

Separation of the coccus from the rod-shaped bacterium proved to be somewhat difficult at first. Culture filtrates of the rod-shaped bacterium, various growth factors, and other common organic substrates were tried but resulted in only erratic improvement of growth of the coccus. Finally, a correlation was noted between size of inoculum and the initiation of growth. This suggested that the pH of the medium as usually prepared (7.0 to 7.5) might have been too low and has been shifted to a more favorable level by addition of the large alkaline (pH 8.5 to 9.0) inoculum. Such was the case, for small inocula (0.1 to 1.0 per cent) initiated good growth and abundant gas production in a formate-mineral medium adjusted to pH 8.0 to 8.2, but did not give rise to development in this medium adjusted to pH 7.0 to 7.4. Larger inocula (10 per cent) initiated growth in both media.

Final purification of the coccus was achieved with a mineral-formate medium adjusted to pH 8.0. Microscopic examination of the culture indicated that only motile cocci were present. The formate-decomposing rod-shaped bacterium was shown to be absent by inoculating a yeast extract-formate medium (pH 7.0 to 7.4) and incubating it anaerobically. No growth occurred. No organisms developed in a yeast-extract peptone medium inoculated with the culture and incubated anaerobically, indicating that amino acid fermenting bacteria were absent. During subsequent experiments on the physiology of the coccus, a variety of organic carbon compounds were tested anaerobically. The absence of growth on glucose or common organic acids was evidence that there was no contamination with the ordinary sugar-fermenting bacteria. In formate-agar shake cultures the colonies were all of one kind and contained the coccus. Therefore, the purity of the culture was established.

Evidence that methane is produced from formate by a pure culture of the coccus will be presented in the following paragraphs.

Morphology. The organism is a medium sized coccus, the cells in a single culture varying from 0.5 to 4 μ in diameter (figure 1). Small and very large forms are in the minority; the average is 1 to 2 μ. The cells are often slightly elliptical and frequently occur in pairs. Sometimes one member of the pair is much smaller than the other and resembles a bud on a yeast cell. A few of these can be seen in figure 1. Bacteria from deep agar colonies have very distorted shapes when first removed from the agar but soon assume a spherical form.

The organism is actively motile, and the cocci, either singly or in pairs, describe a spiral path. Flagellation has not been determined.

The cell wall of the coccus appears to be very fragile. In the preparation of dried stained smears, the cells disintegrate and are almost unrecognizable; therefore, microscopic examinations must be made on wet preparations. The addition of methyl cellulose to slow down motility also disrupts the cells. In order to obtain a preparation suitable for photographic purposes, it was necessary to mix melted agar with a drop of liquid bacterial suspension and a drop of stain on a slide.

Deep agar colonies are 0.5 to 1 mm in diameter, lenticular, and light brown in color. The margins are usually regular but may be slightly lobate. Microscopically, the surface of the colony appears granular.

Cultural conditions. The effect of varying the composition of the medium on the growth of the coccus was investigated systematically. Acetate, propionate, butyrate, succinate, glucose, ethyl alcohol, and methyl alcohol were not attacked when tested at a concentration of 0.5 per cent in the mineral-bicarbonate medium (see later) at pH 8.0. In the same experiment, formate supported good growth

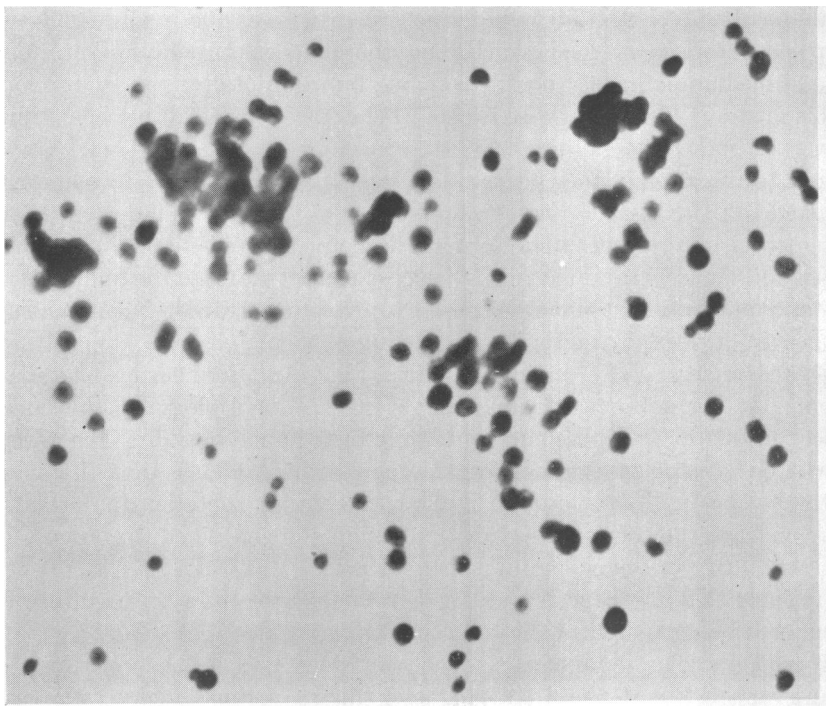

Figure 1. Methanococcus vannielii. Wet preparation of cells from a five day old culture. Erythrosive. × 1700.

Growth, determined turbidimetrically, was studied as a function of sodium formate concentration over the range 0.25 to 5 per cent. Maximum growth [$(2 - \log G) \times 10^3 = 68$] was obtained at a formate concentration of 1.5 per cent. At 2 and 3 per cent levels growth started more slowly, and in 5 per cent sodium formate, the organism failed to develop. By making serial transfers in media containing 3 per cent formate, it was possible to obtain rapid growth also at the level of the substrate.

The substitution of 0.05 to 0.1 per cent $(NH_4)_2SO_4$ for 0.05 per cent NH_4Cl had a slightly beneficial effect on growth as determined by turbidity measurements. Higher concentrations of $(NH_4)_2SO_4$ were inhibitory.

The pH range for growth is between 7.4 and 9.2 with an optimum in the

region of pH 8. During the fermentation of sodium formate, the pH tends to rise rapidly because a relatively strong acid is converted to methane and carbon dioxide, a weak acid. The final pH after growth of the coccus in the medium given below is 9.0 to 9.4. In order to retard the development of alkalinity, tris-(hydroxymethyl)-aminomethane (TRIS) (Gomori, 1946) and glycine buffers were tried at concentrations of 0.01, 0.02, and 0.04 M. The initial pH was 7.5 to 7.7. At the lower concentrations neither buffer was beneficial. At a level of 0.04 M, glycine was slightly inhibitory. TRIS also retarded the initiation of growth about 24 hours, but once growth started, it was more rapid than with added buffer, and the final pH was reduced to 8.8. This buffer should be useful when a large decomposition of the substrate is desired.

Varying the potassium phosphate concentration from 0.01 to 0.04 M had no apparent effect on the rate or amount of growth. The lower level was used in most subsequent experiments.

Carbon dioxide need not be added to the medium to secure normal growth of the coccus, since enough bicarbonate is supplied in a 0.1 volume per cent inoculum. An actual requirement for carbon dioxide has not been demonstrated. The coccus is a strict anaerobe. Oxygen may be removed from the medium by adding either 0.05 per cent sodium thioglycolate or 0.03 per cent $Na_2S \cdot 9H_2O$. An anaerobic seal is used to maintain anaerobic conditions. If a pyrogallol-K_2CO_3 seal is used, the resulting carbon dioxide tends to lower the pH of the medium about 0.5 unit. To compensate for this effect, the initial pH should be increased by a suitable addition of sterile sodium carbonate.

The following medium supports vigorous growth of the coccus: 1.5 per cent sodium formate, 0.1 per cent $(NH_4)_2SO_4$, 0.001 per cent $CaCl_2 \cdot 2H_2O$, 0.001 per cent $MgCl_2 \cdot 2H_2O$, 0.002 per cent $FeCl_3 \cdot 6H_2O$, 0.001 per cent $MnSO_4 \cdot 4H_2O$, 0.0001 per cent $Na_2MoO_4 \cdot 2H_2O$, 0.2 per cent K_2HPO_4, 0.0003 per cent phenol red indicator, 0.0002 per cent methylene blue, 0.05 per cent sodium thioglycolate, and glass distilled or tap water. With the latter, the manganese, molybdate, and iron are probably unnecessary. Agar may be used as desired. If a pyrogallol-K_2CO_3 seal is used, the reaction of the medium after addition of the inoculum should be pH 8.3 to 8.5. When an "oxsorbent" seal is employed, the medium after inoculation is adjusted to pH 7.5 to 8.0. Cultures are incubated at 30 to 40 C.

Fermentation of formate. To determine the products of the fermentation, a pure culture of the coccus was grown in 100 ml of the usual medium containing 1 per cent sodium formate and 0.25 per cent sodium carbonate. The gas evolved during 90 hours' incubation at 30 C was collected over mercury. Both the gas and medium were analyzed.

If formate was converted quantitatively to methane and carbon dioxide, the reaction would be represented by equation (1). The data given in table 1 show that this equation does not describe the fermentation accurately, since hydrogen is formed in addition to methane and carbon dioxide.

$$4HCOONa + H_2O \rightarrow Na_2CO_3 + 2NaHCO_3 + CH_4 \qquad (1)$$

The production of hydrogen causes an increase in the yield of carbon dioxide and a decrease in methane. It should be noted that the analytical data are somewhat inaccurate or incomplete, as indicated by the high carbon recovery (102 per cent) and redox index (1.27). The high redox index is due in part to the synthesis of cell materials that are more reduced than the substrate. The cell material was disregarded in calculating the index.

In preliminary experiments to determine the composition of the evolved gas as a function of the age of the culture, evidence was obtained that hydrogen is produced mainly during the later stages of the fermentation. This suggested a possible correlation between hydrogen production and the pH of the medium.

To establish the facts more clearly, a 20 liter culture of the coccus was grown in the medium previously described. The volume of gas produced during growth was measured by means of a precision gas meter. Gas samples were collected at various stages of the fermentation and their composition determined by the usual methods. Changes in pH were followed with a glass electrode.

TABLE 1

Fermentation of sodium formate

COMPOUND	INITIAL mM/100 ML	FINAL* mM/100 ML	DIFFERENCE mM/100 ML
Formate	12.10	0.30	−11.80
Carbon dioxide	2.64	13.17	10.53
Methane		1.53†	1.53
Hydrogen		1.60†	1.60

* Carbon recovery = 102 per cent; redox index = 1.27.

† Amount in gas collected plus the amount calculated from solubility data to be dissolved in the medium.

Gas evolution started after 14 hours' incubation and continued slowly during the next 24 hours. About this time there was a rapid increase in the rate of gas evolution that was maintained for about 10 hours. Thereafter, the rate fell off sharply and the fermentation appeared to be completed.

The initial pH of the medium was 7.6. After 26 hours' incubation, the pH had risen to 8.6 and the gas was almost pure methane, less than 5 per cent hydrogen being present. At the time when the rate of gas evolution began to increase rapidly (after 38 hours), the pH had risen to 8.75 and the gas was almost 40 per cent hydrogen. After 48 hours, the medium had a pH of 9.0 and the gas was still about 40 per cent hydrogen and 60 per cent methane.

These results indicate that highly alkaline conditions are unfavorable for the production of methane from formate. Instead, much of the formate is decomposed to carbon dioxide and hydrogen. The formation of considerable amounts of hydrogen later in the fermentation accounts for the rapid increase in rate of gas evolution that was observed. Whereas the conversion of formate to methane (reaction 1) gives one-fourth of a mole of water-insoluble gas, the conversion to

hydrogen (reaction 2) yields one mole. Thus a fourfold increase in gas production results from the shift from methane to hydrogen evolution.

$$HCOONa + H_2O \rightarrow NaHCO_3 + H_2 \quad (2)$$

The conversion of much of the substrate to carbon dioxide and hydrogen as the pH of the medium approached 9.0 also explains, in part, why growth is so much poorer in this range. The energy yield of this reaction is very low, and consequently the organism derives little value from the substrate that is decomposed.

Manometric studies with cell suspensions. The coccus was harvested by centrifugation from 1 liter and 20 liter cultures grown in the medium previously described. The suspensions so obtained were usually very slimy in character, a large percentage of the cells having been ruptured.[3] This occurred during centrifugation at 2,000 rpm in an International no. 2 centrifuge as well as at higher speeds (30,000 rpm) in a Sharples super centrifuge. However, rapid conversions of the substrates were catalyzed even by very slimy preparations. Because of the extensive rupturing of the cells, they were resuspended in 0.03 per cent $Na_2S \cdot 9H_2O$ solution without being washed. The pH of such suspensions is 8.5 to 9.0. If not used immediately, the suspensions were stored at 0 C *in vacuo*. Under these conditions they retain most of their activity for at least 4 days.

Cell suspensions were tested for their ability to catalyze the reduction of carbon dioxide by molecular hydrogen. A rapid hydrogen uptake was observed (figure 2). In contrast to results obtained by Barker (1943) with cells of *M. omelianskii*, the maximum rate of gas uptake was established immediately.

The initial rate was more rapid in the sample at pH 6.8 to 7.0 (curve 1, figure 2) than at pH 8.1 (curve 2, figure 2). However, after 90 minutes the rates of hydrogen uptake were the same for both samples, probably because they both developed the same final pH of 9.0.

At the end of the experiment, the volatile acid content of the samples was determined. If all of the hydrogen had been used to reduce carbon dioxide to formate the reverse of reaction 2, sample no. 1 should contain 150 μM of formate, and sample 2 should contain 99μM. Actually, sample no. 1 was found to contain only 32μM and sample 2, 29μM. Since 9μM of formate were present in the cell suspension initially, 23 and 20μM of formate, respectively, had been produced during the experiment. In view of these results, it is concluded that the main reaction was a reduction of carbon dioxide to methane. Subtracting the amount of hydrogen used in each sample to produce formate (1 μM/μM formate), it can be calculated that 43 μM of methane were formed in sample no. 1 and 26 μM in sample no. 2. A similar decline in rate of carbon dioxide reduction to methane

[3] It is of interest to note that viable cultures of the coccus were obtained recently from 1½ year old dried cells (kept at −10 C) prepared by vacuum drying such a cell suspension in a desiccator over $CaCl_2$. This is especially surprising because this particular lot of dried cells exhibited almost no activity when tested immediately after preparation on a CO_2-H_2 mixture. Since this methane bacterium, like the others so far isolated, is rather difficult to keep viable as a growing culture, it would be more convenient to keep it in the dried state.

with increasing pH has been found (Barker, 1943) with cell suspensions of *M. omelianskii.*

Cell suspensions of the coccus were tested for activity on sodium formate in a nitrogen atmosphere. The initial pH was 8.1. Gas production started immediately and continued at a constant rate until 29 μM of gas had been produced. At this point, the reaction stopped completely. The final pH of the sample was 8.8, and all of the formate had been consumed. The sample initially contained 20 μM of added formate, and about 9 μM were present in the cell suspension. Thus one mole of gas was produced per mole of formate decomposed. A conversion of

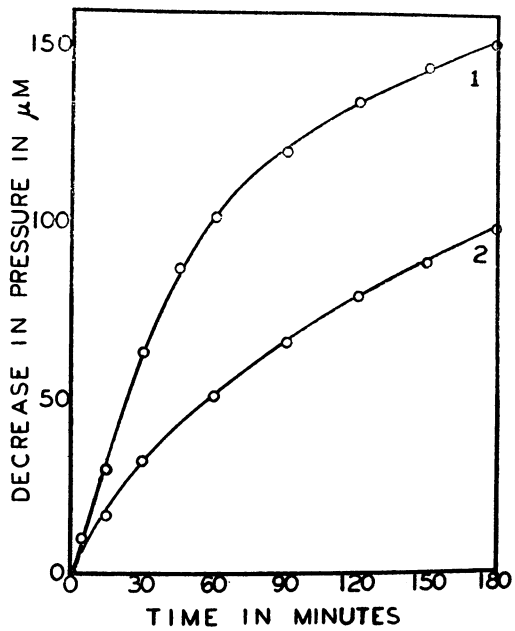

Figure 2. Carbon dioxide reduction by molecular hydrogen. Each Warburg vessel contained 1.0 ml of a bacterial suspension representing cells from 1 liter of culture medium, 0.02 per cent $Na_2S \cdot 9H_2O$, 0.005 M added $NaHCO_3$, and 0.05 M potassium phosphate buffer, pH 6.8 (curve 1) or pH 8.1 (curve 2). Total volume 2.0 ml. Gas phase, hydrogen. Temperature, 37 C.

formate to carbon dioxide and hydrogen would account for this 1:1 ratio since above pH 8.0 the amount of carbon dioxide in the gas phase is negligible. The gas could not have been methane because the theoretical ratio of methane formed to formate decomposed is 0.25.

Experiments were conducted to test the effect of pH on formate decomposition over the range 6.8 to 8.1. Two vessels were employed at each pH level; in one the total pressure changes were measured (carbon dioxide and hydrogen or methane) and in the other KOH was added to the center well to remove the carbon dioxide. The difference in pressure between the two vessels should give an indication as to whether hydrogen or methane was being formed. However,

this technique was not successful. Pressure increases were observed in the absence but not in the presence of alkali. It appears therefore that carbon dioxide is essential for the decomposition of formate even though it is a product of decomposition. Although of considerable interest, this point has not been investigated further.

Rate of C^{14} exchange between $HC^{14}OONa$ and CO_2. Since it had been demonstrated that under certain conditions the coccus decomposes formate to carbon dioxide and hydrogen and also produced the reverse reaction to a slight extent, it seemed likely that there would be an exchange of carbon between carbon dioxide and formate. An experiment was conducted to determine if such an exchange occurs and, if so, how rapidly equilibrium is attained.

A bacterial suspension representing cells from about 10 liters of culture contained in a total volume of 15 ml was used for the experiment. The suspension had been stored for 4 days at 0 C *in vacuo* and although there had been some loss in activity, the cells still took up hydrogen at a fairly rapid rate. The 15 ml cell

TABLE 2

Exchange of C^{14} between $HC^{14}OONa$ and CO_2

INCUBATION TIME	FORMATE		CO_2	
	mM/15 ml	Specific activity counts/min/mM	mM/15 ml	Specific activity counts/min/mM
hours				
0	1.10	76,500	1.10	*1,180
0.5	1.05	67,500	1.15	11,200
1.5	0.90	50,300	1.30	27,300
3.75	0.45	30,900	1.75	38,700
6.75	0.10		2.10	38,400

* The carbon dioxide initially present was nonradioactive. The C^{14} in the 0 time sample is the result of the exchange that occurred during the brief interval required to mix the sample and remove an aliquot for analysis.

suspension also contained 0.1 M phosphate, pH 6.8, about 1.1 mM of $KHCO_3$, and about 1.1 mM of $HC^{14}OONa$ (76,500 counts/min/mM).

The reaction mixture was placed in a large test tube fitted with a ground glass cap through which was sealed a piece of glass tubing that reached below the surface of the liquid. The other end of the glass tube, extending outside the cap, was closed off with a stopcock. With this apparatus, samples of the medium could be withdrawn from the tube at intervals without losing carbon dioxide from the gas phase.

Immediately after adding the C^{14}-labeled formate a sample was removed for analysis and the head space in the tube filled with nitrogen. The reaction mixture was incubated at 37 C. The reaction was stopped in aliquots removed for analysis by the immediate addition of 50 per cent CO_2-free NaOH to pH 10.0 or more. Carbon dioxide and formate were recovered and converted to $BaCO_3$ for radioactivity measurements.

The data in table 2 show that the specific activity of the formate decreased

throughout the experiment while the specific activity of the carbon dioxide increased. It it obvious, therefore, that there was an exchange of carbon between the formate and carbon dioxide. The data also show that there was a gradual decline in formate concentration. Thus after 6.75 hours, 91 per cent of the formate had disappeared, and an almost equivalent amount of carbon dioxide had been formed. It is apparent, therefore, that the specific activity of the carbon dioxide at any one time was the result both of the exchange reaction and of the conversion of formate to carbon dioxide. An approximate appraisal of these two effects can be made. For example, during the first 1.5 hours of incubation, 0.2 mM of formate with an average specific activity of 63,400 counts/mM was converted to carbon dioxide. If no exchange had occurred, the resulting 1.3 mM of carbon dioxide would have contained a total of 12,680 counts/min and the specific activity would have been 9,750 counts/min/mM. The observed specific activity was 27,300 counts/min/mM or nearly 3 times as great as that expected for formate decomposition. The excess activity (27,300 − 9,750 = 17,550 counts/min/mM) must have been due to the exchange reaction.

A complete exchange of C^{14} between the 1.1 mM of formate (76,500 counts/min/mM) and the 1.1 mM of carbon dioxide would cause both compounds to have specific activities of 38,200 counts/min/mM. At the end of 3.75 hours of incubation, the specific activities of the formate and the carbon dioxide were approximately the same, indicating that almost complete equilibration had taken place between the two compounds.

DISCUSSION

The formate-fermenting coccus undoubtedly is different from any methane bacterium previously described in sufficient detail to be recognizable. Four coccus-shaped methane bacteria have been reported (Barker, 1936; Schnellen, 1947): *Methanosarcina methanica*, *Methanosarcina barkeri*, *Methanococcus mazei*, and the unnamed micrococcus undoubtedly belonging in the genus *Methanococcus*, described by Groenewege (1920). The formate-fermenting coccus is readily distinguished from these bacteria by the grouping of the cells, motility, growth rate, and substrate specificity. For example, the other cocci are nonmotile and able to ferment acetate, whereas the formate coccus is motile and unable to attack acetate. The few species of motile cocci previously described (Breed *et al.*, 1948) have no physiological resemblance to the formate coccus since they are aerobic organisms that generally grow on complex carbohydrate- or protein-containing media.

Although some taxonomists are inclined to disregard the striking physiological and biochemical peculiarities of the methane-producing bacteria and group them with other aerobic and anaerobic bacteria in strictly morphological genera, we prefer to set these bacteria apart in special morphological-physiological genera. According to this point of view, the formate-fermenting coccus clearly belongs in the genus *Methanococcus* Kluyver and van Niel (Breed *et al.*, 1948). The name *Methanococcus vannielii*, nov. spec., is proposed for this organism in honor of C. B. van Niel, who greatly stimulated research on the methane bacteria by developing the carbon dioxide reduction theory of methane formation.

M. vannielii is completely different morphologically from *Methanobacterium formicicum*, but nevertheless, these two bacteria show a striking physiological similarity. For both organisms, formate is the only organic carbon compound known to support growth, and during fermentation it is converted to methane carbon dioxide, and under some conditions, hydrogen. Cell suspensions of both bacteria catalyze the reduction of carbon dioxide to methane with gaseous hydrogen. Ammonium salts satisfy their nitrogen requirements and neither amino acids nor growth factors are required. The only physiological difference we have noted is in the pH range for growth. *M. vannielii* prefers a somewhat more alkaline medium than *M. formicicum*.

It is noteworthy that *M. vannielii* grows more rapidly than any methane-producing bacterium studied up to this time. For this reason and also because the organism yields very active cell suspensions, it appears to be well adapted for the study of the biochemistry of the methane fermentation.

The tracer experiments were originally undertaken with the object of finding out whether methane is formed by direct reduction of formate or by oxidation of formate to carbon dioxide, followed by reduction of carbon dioxide to methane. However, the rapid exchange of carbon between formate and carbon dioxide prevented us from obtaining a simple answer to this question by the tracer method. In this connection it may be noted that a very rapid equilibration of the carbon in formate and carbon dioxide has also been observed with dried cells of *Methanobacterium omelianskii* in unpublished experiments. A preferential inhibition of this reaction by cyanide could not be obtained; the reduction of carbon dioxide was inhibited to a greater extent than the exchange.

SUMMARY

The isolation and culture of *Methanococcus vannielii*, nov. spec., are described. The bacterium is a motile coccus that ferments formate and appears to be unable to use any other organic compound as an energy source. Formate is converted to methane, carbon dioxide, and hydrogen. Cell suspensions of the bacterium catalyze a rapid exchange of carbon between carbon dioxide and formate.

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