JOURNAL OF BACTERIOLOGY, May 1967, p. 1722-1724

Vol. 93, No. 5
Printed in U.S.A.

Methane Utilization by a Strain of *Rhodopseudomonas gelatinosa*

DAVID WERTLIEB[1] AND WOLF VISHNIAC

Department of Biology, University of Rochester, Rochester, New York 14627

Received for publication 11 January 1967

Two roles of methane in bacterial metabolism have been studied: (i) as an end product of fermentation; (ii) as an electron donor and carbon source in respiration. Anaerobic photosynthetic assimilation of methane is a third possibility, and may contribute to an anaerobic microbial ecology (W. Vishniac, Aerospace Med. **31**:678, 1960). This communication describes results demonstrating methane utilization by a photosynthetic bacterium.

An enrichment culture containing: $(NH_4)_2SO_4$, 0.05%; K_2HPO_4, 0.05%; $MgSO_4 \cdot 7H_2O$, 0.1%; trace metals(W. Vishniac and M. Santer, Bacteriol. Rev. **21**:195, 1957), 0.1 ml per 100 ml of medium; $NaHCO_3$, 0.5%; and adjusted to *p*H 7.0 to 7.2, was inoculated with about 50 g of mud from a river bank per 100 ml of medium. A glass-stoppered bottle was half filled with medium and inoculum. Methane was bubbled through the mixture for 15 min, and the bottle was then quickly stoppered and inverted. After incubation at 30 C under incandescent light, serial transfers were made in the same medium (25 ml per 125-ml Erlenmeyer flask) and incubated (30 C, light) under an atmosphere of 5% CO_2 + 95% CH_4. $NaHCO_3$ was added aseptically to make a final concentration of 6.6×10^{-3} M. After several serial transfers, it was necessary to supplement the medium with 0.01% yeast extract to support growth.

Plates of the above medium solidified with 1.5% agar were streaked from a liquid culture and incubated under CO_2 + CH_4 in the light. Only one colony type grew on these plates. A clonal culture was established by serial streaking from isolated colonies. The isolated organism was maintained in liquid medium and identified as *Rhodopseudomonas gelatinosa* by comparison with an authentic strain of *R. gelatinosa* (ATCC 11169). This identification is based on: (i) morphology of cells and colonies; (ii) ability to hydrolyze gelatin; (iii) ability to use citrate as a substrate for growth; and (iv) bacteriochlorophyll and carotenoid content observed on thin-layer sucrose chromatograms (B. Colman and W. Vishniac, Biochim. Biophys. Acta **82**:616, 1964) developed with 10% benzene in hexane.

The defined medium of J. Siegel and M. Kamen (J. Bacteriol. **59**:693, 1950) in distilled water supplemented with trace metals (0.4 ml per 100 ml of medium) supported growth with the substrates listed in Table 1. Utilization of propionate distinguishes this strain of *R. gelatinosa* from the strains described by van Niel (Bacteriol. Rev. **8**:1, 1944). A check of the vitamins required for growth in the defined medium confirmed identification of the bacterium. Thiamine·HCl (0.4 mg/100 ml) and biotin (0.5 μg/100 ml) were both needed for growth (S. Hutner, J. Gen. Microbiol. **4**:286, 1950).

In the absence of any additional organic carbon source, growth under 5% CO_2 + 95% CH_4 was poor (about 10% of the yield obtained when malate was included in the medium). An atmosphere of CO_2 + H_2 supported growth of this bacterium with yields ranging from 20 to 50% of the yield obtained with malate (Table 2). No stimulation of growth under CO_2 + CH_4 was observed by changing the *p*H or varying the concentration of trace metals, vitamins, nitrogen source, CO_2, or CH_4.

Table 3 summarizes the results of a series of experiments which attempt to demonstrate CH_4 fixation directly. Cells harvested from mass culture were resuspended in 140 ml of the defined medium supplemented with 6.6×10^{-3} M HCO_3^- in a double-neck, round-bottom, 300-ml boiling flask. The flask was fitted with a three-way stopcock and a break-seal vial containing 100 μc of $^{14}CH_4$ (specific activity, 5.00 mc/mmole). The $^{14}CH_4$ was added to an anaerobic atmosphere of 5% CO_2 + 95% CH_4 in the flask. At the end of the incubation, the flask was evacuated and flushed four times with N_2. The effluent gases were passed through a combustion train (700 C), a trap cooled to −40 C to remove water vapor, and a series of six traps (each containing 10 ml of the trapping medium) to collect CO_2. Carbon dioxide was trapped and radioactivity was deter-

[1] Predoctoral Fellow of the U.S. Public Health Service.

TABLE 1. *Substrate utilization by a strain of Rhodopseudomonas gelatinosa*

Substrate, 0.2%	Growth[a]	
	Without added HCO_3^-	With 6.6×10^{-3} M HCO_3^-
Na-malate	1.15	1.14
Na-lactate	1.20	1.24
Na_2-succinate·$6H_2O$	0.68	0.68
Na-propionate	0.29	1.22
Na-acetate·$3H_2O$	0.80	0.74
Na-formate	0.10	0.38
Ethyl alcohol	0.14	1.15
n-Propanol	0.12	1.32
n-Butanol	0.16	1.17

[a] Expressed as optical density at 680 mμ after 5 days in the light in filled screw-cap test tubes.

TABLE 2. *Growth of Rhodopseudomonas gelatinosa under different atmospheres*

Conditions of incubation[a]	Growth[b]
Under 5% CO_2 + 95% CH_4	0.12
Under 5% CO_2 + 95% H_2	0.45
Under 5% CO_2 + 95% N_2	0.05

[a] Cells were grown in 25 ml of the maintenance medium supplemented with 6.6×10^{-3} M HCO_3^-.

[b] Expressed as optical density at 680 mμ after 6 days of growth in the light in 125-ml Erlenmeyer flasks.

mined by the methods of H. Jeffry and J. Alvarez (Anal. Chem. **33**:612, 1961). After the cells were collected and washed once, they were extracted and separated (D. Hoare, Biochem. J. **87**:284, 1963) into three fractions: (i) water-soluble; (ii) acetone-soluble, water-insoluble; (iii) cell residue. Samples of each fraction were degraded to CO_2 which was trapped and counted as above.

Low but significant levels of incorporation were detected. Experiments 1 and 2 show that the labeling is dependent on live cells, and experiments 3 and 4 show incorporation to be light-dependent. An attempt to increase the specific activity of the labeled fractions by incubation under $^{14}CH_4$ in the absence of carrier CH_4 was not successful.

The medium used in the above incubation experiments, after removal of the cells, contained significant levels of radioactivity. To determine how much of this labeling was due to retention of $^{14}CH_4$, samples of the media were treated as follows: (i) *p*H was adjusted to >8.3; (ii) 15% $BaCl_2 \cdot 2H_2O$ was added in excess; (iii) samples containing barium precipitate were heated to boiling and cooled for 15 min, in a continuous stream of N_2; the effluent gas was passed through toluene to trap any CH_4; (iv) barium precipitate was collected, washed with hot water and ethyl alcohol, and dried; (v) barium precipitate was suspended in water, the *p*H was adjusted to <3.8, and the suspension was treated as in step iii

TABLE 3. *$^{14}CH_4$ fixation by a Rhodopseudomonas gelatinosa strain*[a]

Expt	Conditions of incubation	Cell material in incubation (mg, dry wt)	CH_4 incorporated (μmoles/mmole of CH_4 added)	Total dpm in cell fractions			Total dpm in cell-free medium	
				Water-soluble	Acetone-soluble, water-insoluble	Cell residue	As $^{14}CH_4$	As $^{14}CO_2$
1[b]	Light; under CO_2 + CH_4 + $^{14}CH_4$; 24 hr	327	1.4×10^{-1}	2.87×10^3	1.37×10^3	27.5×10^3	ND	ND
2[b]	Repeat of 1 with killed cells	335	—	NS	NS	NS	ND	ND
3[c]	Light; under CO_2 + CH_4 + $^{14}CH_4$; 24 hr	56.1	9.6×10^{-2}	NS	4.44×10^3	16.9×10^3	15.2×10^3	18.3×10^3
4[c]	Dark, under CO_2 + CH_4 + $^{14}CH_4$; 24 hr	135	4.2×10^{-3}	NS	NS	0.94×10^3	15.2×10^3	27.9×10^3

[a] NS = not significantly > background; ND = not determined.

[b] Cells for these experiments were grown in the defined medium supplemented with 6.6×10^{-3} M HCO_3^- + 0.3% L-malic acid under 5% CO_2 + 95% N_2.

[c] Cells for these experiments were grown in the maintenance medium supplemented with 6.6×10^{-3} M HCO_3^- under 5% CO_2 + 95% CH_4.

except that the effluent gas was passed through the CO_2-trapping medium used for degradations; (vi) samples of the toluene (containing $^{14}CH_4$) and the trapping medium (containing $^{14}CO_2$) were counted.

As seen in the last two columns of Table 3, most of the radioactivity found in the cell-free medium occurs as CO_2. These results suggest that some $^{14}CH_4$ has been oxidized to $^{14}CO_2$, and that the reaction is not stimulated by the presence of light

Ability of the bacterium described here to grow with CH_4 as the sole electron donor has not been demonstrated unequivocally. This organism can, however, (i) incorporate CH_4 carbon into cellular components, and (ii) oxidize CH_4 to CO_2.