APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1986, p. 1436–1437
0099-2240/86/121436-02$02.00/0

Vol. 52, No. 6

Methane Production from Formate by Syntrophic Association of *Methanobacterium bryantii* and *Desulfovibrio vulgaris* JJ

JEAN-PIERRE GUYOT†* AND ALAIN BRAUMAN

Laboratoire de Microbiologie and Institut Français de Recherche Scientifique pour le Developpement en Coopération, Université de Provence, 13331 Marseille Cedex, France

Received 15 May 1986/Accepted 19 September 1986

Coculture of a sulfate-reducing bacterium, when grown in the absence of added sulfate, with *Methanobacterium bryantii*, which uses only H_2 and CO_2 for methanogenesis, degraded formate to CH_4. A pure culture of *Desulfovibrio vulgaris* JJ was able to produce small amounts of H_2. Such a syntrophic relationship might provide an additional way to avoid formate accumulation in anaerobic environments.

It was previously shown that formate can inhibit the acetoclastic reaction in the presence of *Methanosarcina barkeri* 227 or *Methanosarcina barkeri* subsp. *thermophila* (5). In the same paper, a hypothesis was postulated that formate could be transformed to CH_4 in sulfate-depleted environments through interspecies hydrogen transfer.

Sulfate-reducing bacteria (SRB) cannot use formate as an energy source without electron acceptors, as for ethanol or lactate. The use of ethanol or lactate by SRB in sulfate-depleted environments is possible only through an interspecies hydrogen transfer to produce methane (3, 10). This paper reports the production of methane in the absence of added sulfate by coupling an SRB with a hydrogenophilic bacterium unable to use formate.

Techniques described by Hungate (7) and Balch et al. (1, 2) were used throughout this study. *Desulfovibrio vulgaris* JJ, isolated from estuarine sediments, was a gift of W. J. Jones, University of Georgia. *Methanobacterium bryantii* DSM 863 was purchased from the DSM Collection, Göttingen, Federal Republic of Germany.

D. vulgaris JJ was cultivated at 37°C in a previously described medium (8), except that formate was used as substrate (20 mM) with 5 mM sulfate. *D. vulgaris* was inoculated at the end of the exponential phase to obtain the lowest residual concentration of sulfate in the inoculum. *Methanobacterium bryantii* DSM 863 was cultivated at 37°C in medium 1 of Balch et al. (1), in the presence of H_2-CO_2 (80:20).

All experiments were carried out in triplicate in 60-ml serum bottles, each containing 20 ml of the Balch et al. (1) medium 1 prepared without sulfate. It was checked that *Methanobacterium bryantii* was unable to produce methane from formate in pure culture under the experimental conditions used. Formate was determined colorimetrically as described by Lang and Lang (9). Liquid samples (0.5 ml) for formate analysis were removed aseptically, acidified with 10 μl of H_3PO_4 (50%), and centrifuged at 12,000 × *g* for 10 min. Methane was analyzed as previously described (6). Hydrogen was analyzed with a Girdel chromatograph equipped with a thermal conductivity detector, using a 1.80-m Carbosphere (60/80 mesh; Interchim, France) column operated at 85°C. Gas sampling was done with a gastight pressure lock syringe.

After 90 h of incubation, *D. vulgaris*, when inoculated in a medium without sulfate but in the presence of formate, produced 3.7 μmol of H_2 per ml of liquid phase without any detectable growth. This value was very low compared with the theoretical amount of hydrogen (17.5 mmol) which could be evolved from the added formate. The coculture of *D. vulgaris* and *Methanobacterium bryantii* produced methane from formate (Fig. 1): in part I of Fig. 1, 17.5 mmol of formate was converted to 2.5 μmol of CH_4 per ml of liquid phase after 40 h of incubation. The yield, compared with the expected methane production ($4HCO_2^- \rightarrow 1CH_4$), was 57%. The hydrogen needed to produce this amount of methane was 10 μmol/ml of liquid phase, calculated from the equation $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ (13). This was much higher than the amount of hydrogen produced by *D. vulgaris* alone. After 80 h of incubation, 17.5 mmol of formate was added (Fig. 1, part II), and 20 h later all formate had been

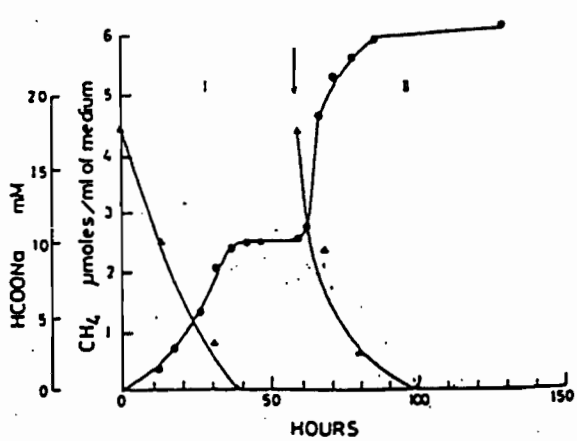

FIG. 1. Methane production from formate in the absence of added sulfate by the coculture of *D. vulgaris* JJ and *Methanobacterium bryantii*. (I) CH_4 production from 17.5 mmol of formate added at the beginning of the experiment; (II) CH_4 production from 17.5 mmol of formate added 60 h later. Symbols: ●, CH_4; ▲, formate.

* Corresponding author.
† Present address: Universidad Autonoma Metropolitana Iztapalapa, Departamento de Biotecnologia, Iztapalapa, 09340 Mexico DF.

TABLE 1. Different reactions by which formate can be transformed in anaerobic environments

Reaction	Reducing microorganisms	Reference
$4HCO_2^- + SO_4^{2-} + H^+ \rightarrow 4HCO_3^- + HS^-$	SRB	13
$HCO_2^- + H_2O \rightarrow H_2 + HCO_3^-$	*Escherichia coli*	5
$4HCO_2^- + H^+ \rightarrow CH_3CO_2^- + 2HCO_3^-$	*Acetobacterium woodii*	
$4HCO_2^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^-$	*Methanobacterium formicicum*	13

utilized, producing 3.5 μmol of methane per ml of liquid phase, demonstrating that formate can be used to produce methane through interspecies hydrogen transfer. The level of CH_4 produced, compared with the expected value, was 75%. The fact that this value is higher than the former percentage of 57% might be due to residual sulfate, which might divert a small part of formate toward sulfate reduction at the beginning of the experiment.

The results indicate that formate could be converted to methane, by interspecies hydrogen transfer between SRB and hydrogenophilic methanogens, as follows: (i) half reaction completed by SRB, $4HCO_2^- + 4H_2O \rightarrow 4HCO_3^- + 4H_2$ ($\Delta G^{\circ\prime} = +5.2$ kJ) (13); (ii) half reaction completed by *Methanobacterium bryantii*, $4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ ($\Delta G^{\circ\prime} = -135.6$ kJ); and (iii) sum of these reactions, $4HCO_2^- + H_2O + H^+ \rightarrow CH_4 + 3HCO_3^-$ ($\Delta G^{\circ\prime} = -130.4$ kJ). Since formate, like hydrogen, can be produced by various microorganisms in anaerobic environments, then besides the strategies already known (Table 1), a new one to prevent formate buildup would be available. In sulfate-depleted environments, conversion of formate might be achieved through interspecies hydrogen transfer between SRB and methanogens, as for lactate or ethanol (3, 10). In such environments, methanogens using formate and SRB coupled to methanogens using H_2 could act as an efficient buffering system to prevent formate accumulation, since formate can inhibit some aceticlastic methanogens (5). From this point of view, the extreme specialization of the microflora in an anaerobic digestor is remarkable: the hydrogen- and formate-using methanogens which could be coupled to SRB from one part and the aceticlastic methanogens unable to use formate from another part. Such a specialization is of great interest, since acetate is the major methane precursor in such environments (12).

Furthermore, the results described above could support a hydrogen-cycling mechanism for formate metabolism by SRB, as proposed by Odom and Peck (11). Since hydrogen from formate can be evolved by *D. vulgaris* for use through interspecies hydrogen transfer to produce methane, one might think that in the presence of sulfate, hydrogen too could be evolved by *D. vulgaris* for sulfate reduction and then, according to this mechanism, enough energy would be available through a proton gradient for the synthesis of ATP.

LITERATURE CITED

1. Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: reevaluation of a unique biological group. Microbiol. Rev. 43:260–296.
2. Balch, W. E., and R. S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-COM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. Appl. Environ. Microbiol. 32:781–791.
3. Bryant, M. P., L. L. Campbell, C. A. Reddy, and R. Crabill. 1977. Growth of *Desulfovibrio* in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. 33:1162–1169.
4. Gottschalk, G. Bacterial fermentations, p. 195. *In* M. P. Starr (ed.), Bacterial metabolism. Springer-Verlag, New York.
5. Guyot, J. P. 1986. Role of formate in methanogenesis from xylan by *Cellulomonas* sp. associated with methanogens and *Desulfovibrio vulgaris*: inhibition of the aceticlastic reaction. FEMS Microbiol. Lett. 34:149–153.
6. Guyot, J. P., I. Traore, and J. L. Garcia. 1983. Methane production from propionate by methanogenic mixed culture. FEMS Microbiol. Lett. 26:329–332.
7. Hungate, R. E. 1969. A roll tube method for the cultivation of strict anaerobes, p. 117–132. *In* J. R. Norris and D. W. Ribbons (ed.), Methods in microbiology, vol. 3B. Academic Press, Inc., New York.
8. Jones, W. E., J. P. Guyot, and R. S. Wolfe. 1984. Methanogenesis from sucrose by defined immobilized consortia. Appl. Environ. Microbiol. 47:1–6.
9. Lang, E., and H. Lang. 1972. Spezifsche Farbreaktion zum direkten Nachweis der Ameisensaure. Z. Anal. Chem. 260: 8–10.
10. MacInerney, M. J., and M. P. Bryant. 1981. Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation. Appl. Environ. Microbiol. 41:346–354.
11. Odom, J. M., and H. P. Peck. 1984. Hydrogenase, electron-transfer proteins, and energy coupling in the sulfate-reducing bacteria *Desulfovibrio*. Annu. Rev. Microbiol. 38:551–592.
12. Smith, P. H., and R. A. Mah. 1966. Kinetics of acetate metabolism during sludge digestion. Appl. Microbiol. 14:368–371.
13. Thauer, R. K., K. Jungermann, and K. Decker. 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41:100–180.