Methane Production from Formate by Syntrophic Association of Methanobacterium bryantii and Desulfovibrio desulfuricans in streambed sediments

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Abstract: Coculture of a sulfate-reducing bacterium, when grown in the absence of added sulfate, with Methanobacterium bryantii, which uses only H₂ and CO₂ for methanogenesis, degraded formate to CH₄. A pure culture of Desulfovibrio desulfuricans was able to produce small amounts of H₂. Such a syntrophic relationship might provide an additional way to avoid formate accumulation in anaerobic environments. Decreasing of Formate in revering environment is a necessary process for increased activity of sulfate-reducing bacteria that play an important role in deleting of element's pollutants.

Key words: Methanogenesis, Syntrophic relationship

1- Introduction
Formate can inhibit the acetoclastic reaction in the presence of Methanosarcina barkeri 227 or Methanosarcina barkeri subsp. Thermophila (Guyot, 1986). A hypothesis has postulated that formate could be transformed to CH₄ 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 sulfatedepleted environments is possible only through an interspecies hydrogen transfer to produce methane (Bryant, Campbell, Reddy and Crabill, 1977; Macnerny and Bryant, 1981). 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, 1969, Balch et al., 1976, Guyot, 1986 and Guyot and Traore, 1983 were used throughout this study.

2- Methodology
Desulfovibrio desulfuricans has isolated from streambed sediments of Kashkan river (southwest of Iran, near to Afrineh village in Lorestan province). Methanobacterium bryantii DSM 863 was purchased from the DSM Collection, Gottingen in Federal Republic of Germany. Desulfovibrio desulfuricans was cultivated at 37°C in medium of Jones et al. (Jones, Guyot and Wolfe, 1984), except that formate was used as substrate (20 mM) with 5 mM sulfate. D.desulfurican was inoculated at the end of the exponential phase to obtain the lowest residual concentration of sulfate in the inoculums. Methanobacterium bryantii DSM 863 was cultivated at 37°C in medium 1 of Balch et al., 1979, in the presence of H₂-CO₂ (80:20). All experiments were carried out in triplicate in 60 ml serum bottles, each containing 20 ml of the Balch et al., 1979, 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 calorimetrically as described by Lang and Lang (Lang and Lang, 1972). Liquid samples (0.5 ml) for formate analysis were removed aseptically, acidified with 10 µL of H₃PO₄ (50%), and centrifuged at 12,000×g for 10 min. Methane was analyzed according to Guyot et al. method (Guyot and Traore, 1983). Hydrogen was analyzed with a Girdel chromatograph equipped with a thermal conductivity detector, using a 1.80-m Carbosphere (60/80 mesh) column operated at 85 °C. Gas sampling was done with a gastight pressure lock syringe.

3- Discussion and conclusions

After 90 h of incubation, D. desulfuricans, when inoculated in a medium without sulfate but in the presence of formate, produced 3.7 µmol of H₂ per ml of liquid phase without any detectable growth. This value (3.7 µmol of H₂ per ml of liquid phase) is very low compared with the theoretical amount of hydrogen (17.5 m mol) which could be evolved from the added formate. The coculture of D. desulfuricans and Methanobacterium bryantii produced methane from formate (Fig. 1); in part I of Fig. 1, 17.5 m mol of formate was converted to 2.5 µmol of CH₄ per ml of liquid phase after 40 h of incubation. The yield, compared with the expected methane production (4HCO₃⁻ + 4H₂O → 4CH₄ + 3H₂O (Thauer, Jungerman and Decker, 1977)), was 57%. The hydrogen needed to produce this amount of methane was 10 µmol/ml of liquid phase, calculated from the equation:

4H₂ + HCO₃⁻ + H⁺ → CH₄ + 3H₂O (Thauer, Jungerman and Decker, 1977). This was much higher than the amount of hydrogen produced by D. desulfuricans alone. After 80 h of incubation, 17.5 m mol of formate was added (Fig. 1, part II), and 20 h later all formate had been 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 CH₄ 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 methanogenesis, as follows:

(i) Half reaction completed by SRB,

4HCO₃⁻ + 4H⁺ → 4HCO₃⁻ + 4H₂ (AG° = +5.2 kJ);

(ii) Half reaction completed by Methanobacterium bryantii,

4H₂ + HCO₃⁻ + H⁺ → CH₄ + 3H₂O (AG° = -135.6 kJ);

And (iii) sum of these reactions,

4HCO₃⁻ + H₂O + H⁺ → CH₄ + 4HCO₃⁻ (AG° = -130.4 kJ).

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

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Reducing microorganisms</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>4HCO₃⁻ + 4H⁺ → 4HCO₃⁻ + 4H₂</td>
<td>SRB</td>
<td>10</td>
</tr>
<tr>
<td>HCO₃⁻ + H₂O → H₂</td>
<td>Escherichia coli</td>
<td>1</td>
</tr>
<tr>
<td>4HCO₃⁻ + 4H⁺ → 4CH₄ + 3H₂O</td>
<td>Acetobacterium woodii</td>
<td>1</td>
</tr>
<tr>
<td>4H₂ + HCO₃⁻ + H⁺ → CH₄ + 3H₂O</td>
<td>Methanobacterium</td>
<td>10</td>
</tr>
</tbody>
</table>

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 build up would be available. In sulfatedepleted environments, conversion of formate might be achieved through interspecies hydrogen transfer between SRB and methanogenesis, as for lactate or ethanol (Bryant et al., 1977; Maclnerney and Bryant, 1981). In such environments, methanogens using formate and SRB coupled to methanogens using H₂ could act as an efficient buffering system to prevent formate accumulation, since formate can inhibit some aceticlastic methanogens (Guyot, 1986). From this point of view, the extreme specialization of the microflora in an anaerobic digester is remarkable. The hydrogen and formate-using methadones, which could be coupled to SRB from one part and the aceticlastic methanogens is unable to use formate from another part. Such a specialization is of great interest, since
acetate is the major methane precursor in such environments (Smith and Mah, 1966). Furthermore, the results described above could support a hydrogen-cycling mechanism for formate metabolism by SRB, as proposed by Odom and Peck (Odom and Peck, 1984). Since hydrogen from formate can be evolved by D. desulfuricans 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. desulfuricans for sulfate reduction and then, according to this mechanism, enough energy would be available through a proton gradient for the synthesis of ATP.

4- References cited


