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Interspecific hydrogen transfer during methanol degradation by *Sporomusa acidovorans* and hydrogenophilic anaerobes

Ralf Cord-Ruwisch and Bernard Ollivier

Laboratoire de Microbiologie ORSTOM, Université de Provence, 3 Place Victor-Hugo, F-13331 Marseille, Cedex 3, France

Abstract. In the presence of active hydrogenophilic sulfate-reducing bacteria, the homoacetogenic bacterium *Sporomusa acidovorans* did not produce acetate during methanol degradation. H_2S and presumably CO_2 were the only end products. Since the sulfate-reducer did not degrade methanol or acetate, the sulfidogenesis from methanol was related to a complete interspecific hydrogen transfer between both species.

In coculture with hydrogenophilic methanogenic bacteria (*Methanobacterium formicicum, Methanospirillum hungatei*), the interspecific hydrogen transfer with *S. acidovorans* was incomplete. Beside CH_4 and presumably CO_2, acetate was produced. The results suggested that H_2-production and H_2-consumption were involved during anaerobic methanol degradation by *S. acidovorans* and the hydrogenophilic anaerobes play an important role during methanol degradation by homoacetogenic bacteria in anoxic environments.

Key words: Methanogenesis – Sulfidogenesis – Homoacetogenesis – Competition for H_2 – *Sporomusa acidovorans* – Interspecies hydrogen transfer

Methanol is formed in nature during the anaerobic degradation of pectin, a major component of plant cell walls (Schink and Zeikus 1981). In anoxic environments, methanol is a typical methanogenic substrate (Oremland et al. 1982). Therefore anaerobic enrichments in the absence of sulfate lead generally to the development of methylotrophic methanogenic bacteria (König and Stetter 1982; Miller and Wolin 1983; Sharak-Genthner et al. 1981).

However in anaerobic upflow reactors fed with methanolic wastes, methanol was partially degraded to acetate (Lettinga et al. 1979, 1981). A sporulating homoacetogen has been shown to be responsible for that reaction (Adamse and Vezeboer 1982).

Anaerobic CH_4 producing enrichment cultures on methanol from a fermenter fed with alcohol distillation wastes contained *Sporomusa acidovorans*, an homoacetogen as predominant methanol-degrader (Ollivier et al. 1985). Attempts to isolate methylotrophic methanogens failed. Therefore, methanogenesis was thought to result from the degradation of acetate, the only endproduct excreted by *S. acidovorans*. But aceticlastic methanogens (*Methanothrix* sp. and *Methanosarcina* sp.) which differ morphologically from all other methanogens were never observed. The predominant methanogenic bacterium in this environment was a rod shaped bacterium, morphologically related to hydrogenophilic *Methanobacterium* species.

These observations indicated that H_2 rather than acetate was the intermediary product during methanogenesis from methanol.

Offprint requests to: R. Cord-Ruwisch

Materials and methods

Sources of organisms

Methanospirillum hungatei (DSM 864) and *Desulfovibrio vulgaris* G6 were isolated from the defined synthrophic association with *Synthrophus bushwellii* (DSM 2612TB). *Methanobacterium formicicum* strain MF and *Methanosarcina* 227 were kindly provided by Prof. R. S. Wolfe, University of Illinois, USA. *Sporomusa acidovorans* was from the collection of our laboratory (DSM 3132).

Medium and growth conditions

The anoxic mineral, bicarbonate buffered, sulfide reduced medium was prepared as described for *Desulfotomaculum sapomandens* (Cord-Ruwisch and Garcia 1985) and supplemented with 0.1% yeast extract (Difco). Stock solutions of methanol were autoclaved separately. Transfers were carried out by sterile syringes.

Chemical determinations

Sulfide was determined photometrically as colloidal CuS (Cord-Ruwisch 1985). Methane, volatile fatty acids and alcohols were analyzed as previously described (Garcia et al. 1982).

Results

Pure cultures of the homoacetogenic bacterium *Sporomusa acidovorans* degrade methanol solely to acetate. In order to verify the assumption that *S. acidovorans* liberates reducing equivalents in the form of hydrogen, during methanol degradation, the strain was grown in coculture with the hydrogen consuming *D. vulgaris* strain G6 which degraded neither methanol nor acetate. H_2S and presumably CO_2 were the only end products of this methanol degrading coculture (Table 1). The degradation of methanol by the coculture was

Table 1
End products of methanol degradation by *Sporomusa acidovorans* in presence and in absence of H_2-consuming methanogenic or sulfidogenic bacteria

	Methanol degraded (mM)	Acetate (mM)	Methane (M) or sulfide (S) (mM)	O/R index
S. acidovorans	15	10.9	0	0.97
S. acidovorans + *Desulfovibrio vulgaris*	10	0	6.9 (S)	0.92
S. acidovorans + *Methanospirillum formicicum*	10	5.7	1.7	0.99
	15	8.9	2.3 (M)	0.99
	20	11.5	2.5	0.93
S. acidovorans + *Methanospirillum hungatei*	10	4.6	3.2	1.04
	15	6.7	4.3 (M)	0.98
	20	8.3	6.4	0.98

Methanol and yeast-extract (0.1%) were the only energy sources. Values are corrected by considering the values of controls containing only yeast-extract. The incubation time was 3 weeks

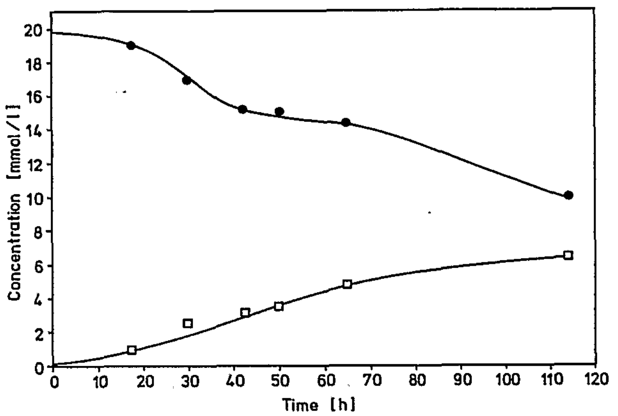

Fig. 1. Time course of methanol degradation by the coculture *Sporomusa acidovorans* — *Desulfovibrio vulgaris*; ●, methanol; □, H_2S

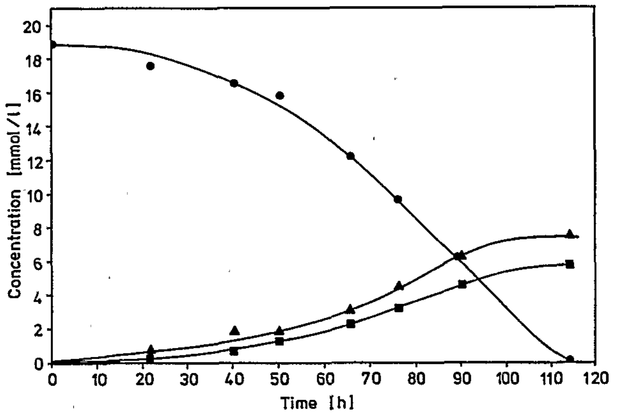

Fig. 2. Time course of methanol degradation by the coculture *S. acidovorans—Methanospirillum hungatei*; ●, methanol; ▲, acetate; ■, methane

not complete. Only about 10 mM methanol was degraded during 3 weeks of incubation (Fig. 1). This inhibition was probably related to the H_2S produced by the *Desulfovibrio* strain since *S. acidovorans* did not grow in the presence of 10 mM H_2S (data not shown).

In coculture with hydrogenophilic methanogenic bacteria, *S. acidovorans* completely consumed methanol (20 mM) without detectable inhibition (Table 1, Fig. 2). Here however, in contrast to the *S. acidovorans—D. vulgaris* coculture, *S. acidovorans* used a part of the reducing equivalents delivered from methanol oxidation to reduce CO_2 to acetate. The percentage of methane produced from methanol was not influenced by the initial substrate concentration but depended on the hydrogenophilic methanogen that was present (Table 1). In the coculture of *S. acidovorans* with *Methanobacterium formicicum*, a smaller part (approx. 20%) of the energy flow from methanol led to methane formation than in the coculture of *S. acidovorans* with *Methanospirillum hungatei* (approx. 40%).

Beside acetate and methane, no other metabolites were observed. The final optical density of the pure culture of *S. acidovorans* and of both methanogenic cocultures was nearly the same (OD = 0.3 at 580 nm) whereas it was less in sulfidogenic cocultures. The growth rate of the methanogenic cocultures on methanol was approximately equivalent to that of *S. acidovorans* grown separately ($t_d = 22$ h). However, *Methanosarcina barkeri* degraded methanol more rapidly ($t_d = 11$ h) than *S. acidovorans.*

Discussion

The intermediary production and consumption of hydrogen which has been presumed for *Methanosarcina* sp. on acetate (Lovley and Ferry 1985) as well as for *Desulfovibrio* sp. on lactate (Odom and Peck 1981) and for the homoacetogenic *Acetobacterium woodii* on fructose (Winter and Wolfe 1980) is probably also involved during the methanol degradation by *Sporomusa acidovorans* which degrades methanol as well as H_2:

$$4\,CH_3OH + 8\,H_2O \rightarrow 12\,H_2 + 4\,HCO_3^- + 4\,H^+ \quad \Delta G'^{\circ} = +94.0 \qquad (1)$$

$$12\,H_2 + 6\,HCO_3^- + 3\,H^+ \rightarrow 3\,CH_3COO^- + 12\,H_2O \quad \Delta G'^{\circ} = -313.8 \qquad (2)$$

$$4\,CH_3OH + 2\,HCO_3^- \rightarrow 3\,CH_3COO^- + 4\,H_2O + H^+ \quad \Delta G'^{\circ} = -219.8 \qquad (3)$$

($\Delta G'^{\circ}$ values obtained from Thauer et al. (1977) and given in kJ/reaction).

This hypothesis was supported by the fact that *S. acidovorans* liberated reducing equivalents in the form of H_2 when cocultured with other H_2-using anaerobes. The energy conserving reaction is due to the oxidation of hydrogen combined with the reduction of CO_2 to acetate

[Eq. (2)]. The presence of other hydrogen consuming bacteria results therefore in competition for hydrogen, produced by the methylotrophic reaction.

D. vulgaris was able to completely outcompete *S. acidovorans* for the hydrogen produced from methanol degradation. All hydrogen produced by the methylotrophic reaction was solely oxidized by the sulfidogen. The first reaction [Eq. (1)], which is endergonic under standard conditions, remains the only possible energy source for the growth of *S. acidovorans*. As explained for obligate hydrogen transferring associations, the hydrogen producing reaction [Eq. (1)] becomes exergonic when the H_2-concentration is kept at a low level (McInerney and Bryant 1980; Thauer et al. 1977). This explains the growth of *S. acidovorans* on methanol even if all the liberated hydrogen is consumed by the sulfate-reducing bacterium.

The methanogenic bacteria which have a lower affinity to hydrogen than sulfate-reducing bacteria (Kristjansson et al. 1982; Lovley et al. 1982) could not completely outcompete *S. acidovorans* for the hydrogen produced from methanol: beside methane, also acetate was produced in methanogenic cocultures on methanol. In coculture with *S. acidovorans*, *Methanospirillum hungatei* was more successful in removing hydrogen (approx. 40%) than *Methanobacterium formicicum* (approx. 20%). This may be due to different hydrogenase-affinities of these methanogens. In the described coculture, *S. acidovorans* oxidized the intermediary hydrogen more effective than both methanogenic bacteria ($\Delta G'^{\circ}$ = −26.15 and −33.9 kJ/mol H_2 respectively). This could be explained by the raised partial pressure of H_2 near by the membranes of the *S. acidovorans*-cells from where it is produced.

S. acidovorans had a disadvantage from the presence of other hydrogenophilic bacteria due to the decrease of its finally formed biomass. Therefore the character of the described H_2-transfering association is more competitive or parasitic than symbiotic.

Despite of its slow growth on methanol, *S. acidovorans* developed in methanol enrichments. This was possibly due to the high concentration of glycerol, one of the favorite substrates of *S. acidovorans* (Ollivier et al. 1985) in the fermenter from where the inoculum originated.

In natural anaerobic environments, the activity of hydrogenophilic methanogens or sulfidogens could reduce the production of acetate from methanol or possibly also from other homoacetogenic substrates. The reduction of CO_2 by homoacetogenic bacteria using different substrates should be tested in the presence of hydrogenophilic methanogenic or sulfidogenic bacteria.

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