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Short Communication

Interspecies hydrogen transfer between the rumen ciliate *Polyplastron multivesiculatum* and *Methanosarcina barkeri*

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Methanogenesis is an important metabolic activity in anaerobic ecosystems such as the rumen. The transfer of hydrogen between fermentative hydrogen producers and methanogens, facultative interspecies hydrogen transfer, provides a sink for hydrogen which would otherwise accumulate and inhibit fermentation (Wolin, 1975). Interspecies hydrogen transfer has been demonstrated between methanogens and a variety of ruminal bacteria and chytridiomycetes (Miller, 1995), and has been shown to stimulate fibre degradation by the chytridiomycetes (Bauchop and Mountfort, 1981; Marvin–Sikkema et al., 1990; Tanaka et al., 1991). Ciliate protozoa are hydrogen producers and thought to be responsible for a large proportion of the fibre degradation in the rumen (Coleman, 1985). Methanogens have been observed both on the cell surface and in the cytosol of rumen ciliates (Finlay et al., 1994; Vogels et al., 1980). Methanogens increased the growth rate and yield of the non-ruminal anaerobic ciliates *Plagiopyla frontata* and *Metopus contortus* (Frenchel and Finlay, 1991). Interspecies hydrogen transfer has been demonstrated between the rumen ciliate *Isotricha* spp. and the methanogen *Methanosarcina barkeri* (Hillman et al., 1988). *Isotricha* is a soluble sugar utilizer and not thought to be active in ruminal fibre degradation (Williams and Coleman, 1992), thus this protozoon makes a relatively minor contribution to rumen feed digestion. Here, we report on the interaction between the cellulolytic rumen ciliate *Polyplastron multivesiculatum* with *M. barkeri* DSM 800 in long-term co-culturing and its influence on feed degradation in vitro.

A culture of *P. multivesiculatum* was prepared from rumen fluid withdrawn from two Texel sheep weighing approximately 60 kg fitted with permanent rumen cannula. The sheep had previously been defaunated by rumen emptying (Jouany and Sénaud, 1979a) and *P. multivesiculatum* introduced as the sole ciliate. The sheep were fed a daily ration composed of 700 g of lucerne pellets, 300 g of ground maize grain, 100 g of meadow hay, and 50 g of wheat straw. The sheep were fed once a day at 9 am. Water and mineral blocks were available at all times. The animals were allowed at least 6 months to adapt to the diet and fauna before they were used as donors of rumen contents. Ruminal fluid was removed just prior to feeding and strained through a double layer of muslin. Protozoa were separated from the strained rumen fluid (400 ml) and were resuspended in 400 ml of anaerobic Coleman's Simplex type buffer (Williams and Coleman, 1992). This suspension was incubated for 5 h at 39°C with 2 g of soluble starch and 40 mg each of streptomycin, penicillin G, and chloramphenicol. The protozoa were then washed and re-suspended in 100 ml of the same buffer. A portion (10 ml) of this suspension was inoculated into a serum bottle containing Simplex type buffer (15 ml), autoclaved and clarified rumen fluid (25 ml) plus finely ground lucerne hay (50 mg) and ground maize grain (50 mg). The initial gas phase of the bottles was O_2-free carbon dioxide. The number of *P. multivesiculatum* in the bottles was *ca.*150 cells/ml. *M. barkeri* DSM 800 was used to

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Table 1. Apparent gas and short-chain fatty acid production (μmol bottle^{-1} day^{-1}) and substrate degradation (% in 96 h) in incubations of *Polyplastron multivesiculatum* alone or in a co-culture with *Methanosarcina barkeri* DSM 800.

	P. multivesiculatum	*P. multivesiculatum*+*M. barkeri*	
H_2	12.4±1.14	6.6±2.20	*
CH_4	ND	70.8±10.30	—
Formate	25.0±8.89	1.9±0.88	*
Acetate	131.0±11.68	141.0±1.21	NS
Propionate	92.6±4.45	98.3±6.11	NS
Butyrate	17.5±0.19	17.0±0.36	NS
Dry matter loss	42.4±1.31	44.5±3.17	NS

Values are means with standard deviations of two incubations. Values were analysed by Student's *t*-test. *, $p<0.05$; NS, not significant; ND, not detected.

establish a co-culture with *P. multivesiculatum*. *M. barkeri* DSM 800 was obtained from Deutsche Sammlung von Mikroorganismen, Gottingen, Germany. This bacterium was routinely subcultured in modified Balch medium containing 20% of rumen fluid under 206 kPa of H_2/CO_2 (80/20) gas (Morvan, 1995). *M. barkeri* (2 ml of a 48 h-old culture) was added to the serum bottles 2 h after inoculation with *P. multivesiculatum*. Incubation continued for 94 h at 39°C with occasional gentle shaking. Gas was collected in a glass syringe at the end of incubation and immediately analysed by gas chromatography (Jouany and Sénaud, 1979b). Short-chain fatty acids in the culture supernatant were analyzed by gas-liquid chromatography (Jouany, 1982), but formic acid was determined using a formate dehydrogenase kit (Boehringer Mannheim, Mannheim, Germany). Residual substrate was collected by centrifugation (10,000×*g* for 15 min) and exhaustively washed with distilled water before drying for 48 h at 80°C to determine the residual dry matter.

P. multivesiculatum maintained their population density throughout incubation with no difference observed between treatments. The presence of *M. barkeri* decreased hydrogen and formate production in favor of methane, acetate, and propionate production (Table 1). The co-culturing of *P. multivesiculatum* with *M. barkeri* also caused a 5% increase in apparent dry matter disappearance. This is the first experimental demonstration of interspecies hydrogen/formate transfer from a cellulolytic entodiniomorphid ciliate protozoon to a methanogen. It is noteworthy that *M. barkeri* DSM 800 reduced not only apparent hydrogen production by *P. multivesiculatum* by 50%, but also apparent formate production by 90%. *M. barkeri* DSM 800 does not use formate (Hutten et al., 1980), suggesting that the decrease in formate production results from a metabolic change in the ciliate; a shift in the flux of carbon away from formate and acetyl Co-A production by pyruvate formate lyase towards acetyl Co-A production by pyruvate dehydrogenase complex with subsequent reduction of ferredoxin. If this is so, it might suggest that acetyl Co-A production in *P. multivesiculatum* is regulated by the presence of the methanogen through a reduction in the partial pressure of hydrogen in the rumen. The present results were principally in line with many co-culturing experiments using a range of hydrogen/formate-producing organisms and methanogens (Bauchop and Mountfort, 1981; Marvin–Sikkema et al., 1990; Tanaka et al., 1991; Wolin and Miller, 1983). Unlike these previous studies, the in vitro co-culturing between ciliate protozoa and methanogens is complicated by the ingestion and digestion of bacteria by protozoa. Evidently the methanogen susceptible to protozoal predation does not persist in co-culturing with protozoa. *M. barkeri* DSM 800 is the least susceptible to predation by rumen protozoa among several methanogens and an eubacterial strain (Newbold et al., 1996), this helped the establishment of co-culturing in this experiment.

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