HOW MANY RUMEN METHANOGENS ARE THERE?

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For nearly 70 years in ruminant nutrition the loss as methane of gross energy intake by ruminants has been measured, but surprisingly little is known about the populations of methane-producing microorganisms (methanogens) in the rumen. Numbers of methanogens in the cattle rumen are *ca.* 10^8/mL rumen contents, and differ little with time after feeding or with the roughage content of the diet (Leedle and Greening 1982). Methanogens that have been isolated from the rumen and from faeces of ruminants belong to the genera *Methanobrevibacter, Methanomicrobium* and *Methanosarcina* (Miller *et al.* 1986, Wolin and Miller 1997). *Methanobrevibacter* spp. and *Methanomicrobium* spp. are present in high numbers in the rumen (*ca.* 10^8/mL), but the sarcina are present in low numbers (*ca.* 10^3/mL) except in animals fed unusual diets where rumen dilution rates are very low. Interestingly, few of the type species are rumen isolates (Sowers and Schreier 1995). Here we report observations of the size and composition of the populations of methanogens in sheep and cattle from several studies.

An agar medium (Miller and Wolin 1982) was used for enumeration and isolation of methanogens. Methanogens were identified by their autofluorescence at 420nm and production of methane in culture. Methane was determined by gas chromatography with flame ionisation or thermal conductivity detectors. Serum antibodies raised in rabbits were used as 'probes' to indicate possible phylogenetic relatedness of isolates, or of cells in rumen contents, observed using immunofluorescence microscopy (see Conway de Macario *et al.* (1982) for more detail).

In rumen inocula from 12 sheep fed oaten hay, lupin grain and a mineral mix (88:10:2) the size of the methanogen population, estimated from colony counts in the agar medium, varied between sheep from less than 10^5 up to 10^8/mL rumen inoculum. In the culture dilution series, the final dilutions at which methane in the head-space was detectable also varied, from dilutions of 10^5 to more than 10^9/mL rumen inoculum.

From two cattle fed either hay or hay silage, methanogens were isolated from dilutions of 10^5 to 10^8/mL rumen contents. The isolates were cocci, coccobacilli or rods and when they were tested against antisera raised to various methanogens they bound most strongly antibodies to either *Methanobrevibacter ruminantium* M1 or *Methanobacterium formicicum* MF. There was little or no cross-reactivity with antibodies to *Methanobrevibacter smithii* B181 or *Methanobrevibacter arboriphilicus* DC.

From a sheep fed silage the morphology of methanogens that were isolated (in the laboratory of M.J. Wolin, New York State Department of Health) resembled *Methanobrevibacter* spp. Antisera (in the laboratory of A. J. L. Macario, New York State Department of Health) raised against *Mb. ruminantium* M1, *Mb. smithii* PS, ALI, *Mb. arboriphilicus* DH1, AZ, DC, *Methanobacterium bryantii* M.o.H.G, *M. formicicum* MF, *Methanomicrobium mobile* BP, *Methanosarcina mazei* S6 and *Methanosarcina barkeri* MS were used to probe methanogens in rumen fluid samples. Coccobacilli that morphologically resembled *Methanobrevibacter* spp. and strongly bound antibodies to *Mb. smithii PS* were most numerous followed by cocobacilli that strongly bound antibodies to *Mb. arboriphilicus* DH1 and DC. Low numbers of large cocci strongly bound antibodies to *Ms. mazei* S6 and *Ms. barkeri* MS.

Since antibody probes revealed a greater number and diversity of methanogens in the rumen population than did culture techniques, ecological studies of rumen methanogens using culture techniques also should employ complementary techniques such as the use of antibody or oligonucleotide probes.

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