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Characteristics of Methanogens Isolated from Bovine Rumen

TERRY L. MILLER,[1]* M. J. WOLIN,[1] ZHAO HONGXUE,[2] AND M. P. BRYANT[2]

Wadsworth Center for Laboratories and Research, New York State Health Department, Albany, New York 12201,[1] and Department of Dairy Science, University of Illinois, Urbana, Illinois 61801[2]

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Six strains of methanogens were isolated from 10^{-8} and 10^{-9} ml of bovine rumen contents. All strains had the morphologic and physiologic characteristics of *Methanobrevibacter* spp. Four strains required coenzyme M; two did not. Growth of all strains either depended on or was stimulated by a mixture of isobutyric, isovaleric, 2-methylbutyric, and valeric acids. None of the strains reacted with antiserum against the type strain of *Methanobrevibacter ruminantium*.

Lovley et al. (4) reported recently the isolation of two *Methanobrevibacter* strains from high dilutions of bovine rumen contents. One strain had characteristics very similar to those of the type strain, M1, of *Methanobrevibacter ruminantium*. Its major substrates for methanogenesis were H_2 and CO_2, and it required coenzyme M for growth. The other strain did not require the coenzyme, used formate as well as H_2 and CO_2 as substrates for growth and methanogenesis, and did not react with an antiserum against the type strain of *M. ruminantium*.

We have also isolated coenzyme M-requiring and -non-requiring strains of *Methanobrevibacter* from high dilutions of rumen contents. The results presented here extend and generally confirm the observations by Lovley et al. (4).

Isolations were made from rumen contents obtained at the University of Illinois from a fistulated steer on a diet of mixed hay, mainly alfalfa. Samples were filtered through cheesecloth and processed immediately. The dilutions were prepared in anaerobic dilution solution (3). Isolates Z3, Z4, Z6, and Z8 were isolated directly from 10^{-8} ml of rumen contents in roll tubes, using an agar medium with 30% rumen fluid and 80% H_2–20% CO_2 (203 kPa). This medium was essentially the same as that previously described for the isolation of type strain M1 (2). Enrichments containing 10^{-9} ml of rumen contents in the rumen fluid medium were used to isolate strains ZA-4 and ZA-10. The enrichments (10^{-8} ml) were plated in roll tubes with a complex medium containing rumen fluid and the antibiotics cephalothin and clindamycin as described previously for isolation of methanogens from human feces (6).

Analysis of roll tubes with colonies by gas chromatography showed the presence of CH_4 at all dilutions. Methanogens were isolated by picking discrete colonies. After replating and ascertaining that the isolates did not grow in complex SMS medium (5) with 0.5% glucose and 100% CO_2 (101.3 kPa), we characterized the isolates by morphologic and physiologic tests (6).

All isolates were short, gram-positive coccobacilli. Stereoscan electron micrographs of two strains are shown in Fig. 1. All isolates used H_2 and CO_2 as substrates for growth and methanogenesis. Formate was used poorly, and acetate, methanol, and trimethylamine were not used as substrates.

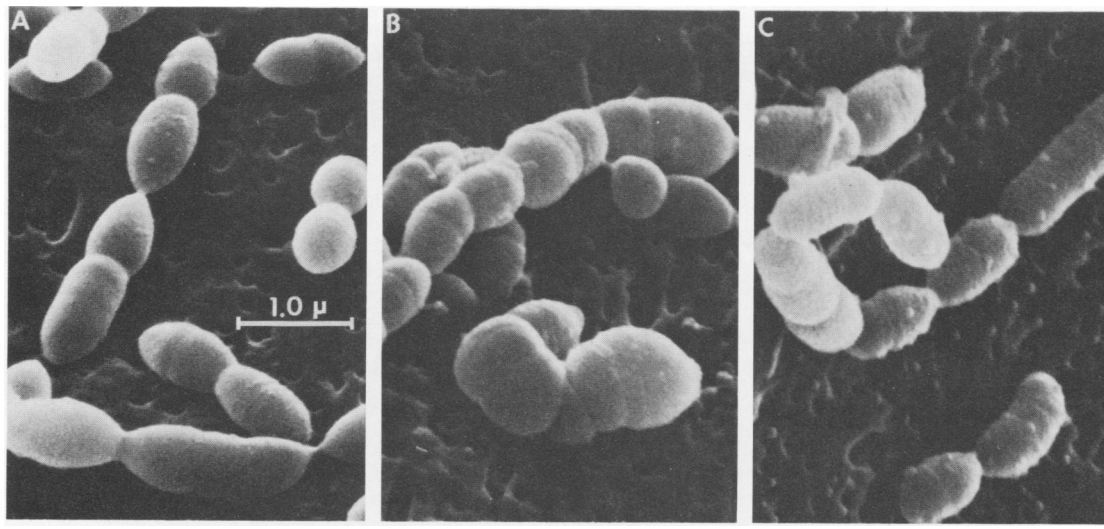

FIG. 1. Scanning electron micrographs of type strain *M. ruminantium* M1 (A) and strains Z4 (B) and ZA-10 (C). Cells were grown in medium 1 of Balch et al. (1), modified to include an additional 1 g of NH_4Cl per liter and 10% (vol/vol) clarified rumen fluid (6). Cells were prepared for scanning electron microscopy as described previously (7).

* Corresponding author.

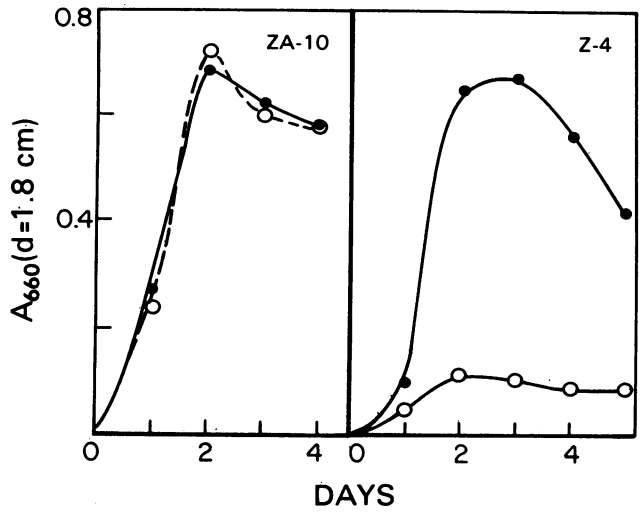

FIG. 2. Growth of strains ZA-10 and Z4 in the presence (●) or absence (○) of coenzyme M. The coenzyme M requirement for growth was examined by using medium 1 of Balch et al. (1) modified to include an additional 1 g of NH_4Cl per liter and the following volatile fatty acids (milliliters per liter): isobutyric (0.54), 2-methylbutyric, *n*-valeric, and isovaleric (0.6 ml each). Coenzyme M was added to a final concentration of 100 μg/liter. The inocula were washed cells from cultures grown in the complete medium with coenzyme M. The depicted growth of strain ZA-10 in the absence of coenzyme M was obtained after four subcultures.

Of six strains examined for coenzyme M requirement, four were found to require the coenzyme, and two grew without it (Fig. 2). Boiled extracts of cells of the two isolates that did not require coenzyme M fully supported the growth of *M. ruminantium* M1 and two of the coenzyme M-requiring isolates. A mixture of four volatile fatty acids—isobutyric, isovaleric, 2-methylbutyric, and valeric acids—was required by one coenzyme M-requiring and one -nonrequiring strain. All other strains were stimulated by the mixture.

These observations confirm that both coenzyme M-requiring and -nonrequiring *Methanobrevibacter* strains are present in high concentrations in bovine rumen contents as reported by Lovley et al. (4). They suggested that the nonrequiring strains may be a species other than *M. ruminantium* because one strain showed no reactivity with a rabbit antiserum against the type strain. However, none of our seven coenzyme M-requiring and -nonrequiring strains reacted with an antiserum against the type strain (E. Conway de Macario, personal communication).

The taxonomic status of the *Methanobrevibacter* spp. isolated from bovine rumen has not been clearly resolved by the physiologic, morphologic, and immunologic tests used thus far. Studies of relationships among macromolecular structures—e.g., DNA, RNA, and cell walls—appear to be necessary for clarification.

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