Nutritional Requirements of *Methanomicrobium mobile*

RALPH S. TANNER[†] AND RALPH S. WOLFE*

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

Received 16 July 1987/Accepted 2 December 1987

A defined medium was developed for *Methanomicrobium mobile* BP. *M. mobile* required acetate for growth; the optimal concentration was 30 mM. Other requirements and their optimal concentrations included isobutyrate (0.65 mM), isovalerate (0.73 mM), and 2-methylbutyrate (1.5 mM). The appropriate branched-chain amino acids did not substitute for these branched-chain fatty acids. *M. mobile* required tryptophan at an optimal concentration of 24 μ M. Indole substituted for tryptophan, but the possible precursor compounds shikimic acid and anthranilic acid and the degradation compound skatole did not. Vitamin requirements and their optimal concentrations included pyridoxine (0.49 μ M), thiamine (0.15 μ M), biotin (0.04 μ M), and vitamin B₁₂ (0.04 μ M); *p*-aminobenzoic acid (0.18 μ M) was required for optimal growth, but folic acid did not replace *p*-aminobenzoic acid. *M. mobile* required an unidentified growth factor found in ruminal fluid or extracts of *Methanobacterium thermoautotrophicum* for growth. *M. mobile* has a complex nutrition compared with that of other methanogens, but not an unusual nutrition in the context of organisms from the ruminal ecosystem.

Methanomicrobium mobile was described in 1968 (17) as a major methanogenic organism in the ruminal ecosystem. Since the initial isolation and characterization, only a limited amount of research has been conducted with *M. mobile* (2, 9, 21), primarily because the organism proved to be one of the most difficult methanogens to culture. *M. mobile* requires a heat-stable growth factor, found in rumen fluid (2, 17), called mobile factor. In addition, the growth of *M. mobile* has been poor compared with that of other methanogens (17). Cultures of *M. mobile* tended to lyse, with loss of viability, after 2 to 4 days of incubation (R. S. Tanner and R. S. Wolfe, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, I90, p. 109). Isolation and characterization of the unknown growth factor have awaited the discovery of a method to reproducibly grow *M. mobile* in stable culture. Since the addition of greater amounts of mobile factor resulted in a more rapid and complete lysis of *M. mobile* (W. E. Balch, personal communication), it was possible that lysis of cultures was due to imbalanced growth. Lysis of other methanogens, including *Methanobacterium bryantii* (10) and *Methanobacterium thermoautotrophicum* (4), has been observed under some growth conditions. In this report we establish the nutritional requirements of *Methanomicrobium mobile* and describe a medium which results in stable, reproducible growth.

MATERIALS AND METHODS

Bacteria and culture conditions. *M. mobile* BP (DSM 1539) was obtained from P. H. Smith, University of Florida, Gainesville, Fla., and maintained by W. E. Balch and R. S. Tanner by frequent transfer in liquid medium. The basal medium was adapted from that of Balch et al. (2) and contained the following, in grams per liter: NH₄Cl, 1.0; (NH₄)₂SO₄, 0.42; KH₂PO₄, 0.42; NaCl, 0.84; MgSO₄ · 7H₂O, 0.18; CaCl₂ · 2H₂O, 0.011; FeSO₄ · 7H₂O, 0.002; resazurin, 0.0001; NaHCO₃, 4.0; cysteine hydrochloride, 0.5; Na₂S · 9H₂O, 0.5. Trace metal solution was added at 10 ml/liter (24). The complex medium was prepared as described by Balch et al. (2) and consisted of the following

additions, in grams per liter, to the basal medium: yeast extract (Difco Laboratories, Detroit, Mich.), 2.0; Trypticase (BBL Microbiology Systems, Inc., Gaithersburg, Md.), 2.0; sodium formate, 2.5; sodium acetate, 2.5; propionic acid, 1.0; butyric acid, 0.6; isobutyric acid, 0.2; valeric acid, 0.2; isovaleric acid, 0.2; 2-methylbutyric acid, 0.2. 2-Mercaptoethanesulfonic acid (100 μ g/liter) and vitamin solution (10 ml/liter) (24) were added. A crude preparation from a cell extract of *Methanobacterium thermoautotrophicum* (as described below) served as the source of mobile factor. *Methanomicrobium mobile* was grown in tubes (18 by 150 mm); each tube contained 5 ml of medium under a pressurized atmosphere of H₂-CO₂ (80:20) (3). The final pH of the medium was 6.9 to 7.1. Tubes were repressurized to 3 atm (300 kPa) at least once every 48 h (usually once each day). Growth was monitored by the method described by Balch and Wolfe (3); each value for growth (*A*₆₆₀) was the average of results from at least three tubes.

Mobile factor. Mobile factor activity was found in boiled cell extract of *Methanobacterium thermoautotrophicum* by W. E. Balch. Crude mobile factor was prepared from the boiled cell extract of *Methanobacterium thermoautotrophicum* by anoxic chromatography on a DEAE-Sephadex A-25 column by the procedure described by Noll et al. (16). Mobile factor was eluted with 150 mM NaCl, and was the peak that eluted after a corrinoid-containing peak and before component B (16). The resulting crude mobile factor had more activity per volume than did boiled cell extract and was handled by using an anoxic technique. Purified mobile factor was prepared from the crude mobile factor by anoxic chromatography on a Dowex 50W-X4 (H⁺ form) column that was equilibrated and eluted with anoxic water. Mobile factor eluted within 2 void volumes. Details of the purification and isolation of mobile factor will be presented elsewhere.

Materials. All of the common chemicals were of reagent grade or better. Yeast extract and vitamin-free Casamino Acids were obtained from Difco. Trypticase was obtained from BBL. Vitamin B₁₂, thiamine hydrochloride, lipoic acid, calcium pantothenate, biotin, riboflavin, folic acid, *p*-aminobenzoic acid (PABA), nicotinic acid, pyridoxine hydrochloride, shikimic acid, indole, skatole, DL-3-indolelactic acid, anthranilic acid, tryptophan, and 2-methylbutyric acid were

* Corresponding author.

[†] Present address: Department of Botany and Microbiology, University of Oklahoma, Norman, OK 73019.

FIG. 1. Growth of *Methanomicrobium mobile* on complex (●) and partially defined (○) media.

obtained from Sigma Chemical Co. (St. Louis, Mo.). 2-Mercaptoethanesulfonic acid was obtained from Pierce Chemical Co. (Rockford, Ill.). Resazurin and isobutyric acid were obtained from Eastman Kodak Co. (Rochester, N.Y.). Isovaleric acid was obtained from Aldrich Chemical Co., Inc. (Milwaukee, Wis.).

RESULTS

The growth of *M. mobile* in the complex medium described by Balch et al. (2) was erratic, and the maximal absorbance of a culture was generally less than 0.2 (Fig. 1). Cultures usually lysed after 2 to 4 days of incubation (Fig. 1). Some improvement in the amount and reproducibility of growth of *M. mobile* was noted after formate and 2-mercaptoethanesulfonate were eliminated from the medium and after the levels of yeast extract and Trypticase were reduced in the medium (data not shown).

Requirement for volatile fatty acids. The effect of the volatile fatty acids present in the complex medium on the growth of *M. mobile* was determined (Table 1). Growth was found to be dependent on acetate, isobutyrate, isovalerate, and 2-methylbutyrate (Table 1). The low level of growth in medium lacking acetate may be explained by the carryover of acetate with the inoculum. The low level of growth in medium lacking isovalerate or 2-methylbutyrate may be explained by the presence of a small amount of contaminating isomer in the preparations of each branched-chain fatty acid. The optimal concentration of each fatty acid for the growth of *M. mobile* was determined to be as follows, in millimolar: sodium acetate, 30; isobutyrate, 0.65; isovalerate, 0.73; 2-methylbutyrate, 1.5. Isobutyrate, isovalerate, and 2-methylbutyrate can be produced from valine, leucine, and isoleucine, respectively, by ruminal bacteria (1). Sources of the branched-chain amino acids (yeast extract, Trypticase, and vitamin-free Casamino Acids) did not substitute for any of the branched-chain volatile fatty acids required by *M. mobile*.

Amino acid requirement. As part of the complex medium, yeast extract (1 g/liter) was required for the growth of *M. mobile*. Vitamin-free Casamino Acids, at 2 g/liter, did not replace yeast extract even when supplemented with a vitamin solution (24), menadione, hemin, Tween 80, adenosine, guanosine, inosine, uridine, thymidine, lactate, pyruvate,

TABLE 1. Effect of volatile fatty acids on the growth of *Methanomicrobium mobile*

Growth condition ^a	Growth (A_{660}) ^b
VFA, complete ^c	0.48
VFA - acetate	0.18
VFA - propionate	0.44
VFA - butyrate	0.48
VFA - isobutyrate	-0.01
VFA - valerate	0.47
VFA - isovalerate	0.11
VFA - 2-methylbutyrate	0.14
No addition	0.00

^a The basal medium consisted of a mineral medium supplemented with yeast extract and Trypticase (1 g/liter each), vitamin solution (24), and crude mobile factor. VFA, Volatile fatty acids.

^b Growth (after 4 days of incubation) was monitored by the method described by Balch and Wolfe (3).

^c Final concentrations of volatile fatty acids in medium, in grams per liter, were as follow: sodium acetate, 2.5; propionate, 1.0; butyrate, 0.6; isobutyrate, 0.2; valerate, 0.2; isovalerate, 0.2; 2-methylbutyrate, 0.2.

succinate, and/or citrate (data not shown). Vitamin-free Casamino Acids are deficient in the amino acids asparagine, glutamine, methionine, and tryptophan (15). It was found that tryptophan (24 μ M) and a vitamin solution (24) could replace yeast extract for the growth of *M. mobile*. Substitution of tryptophan with anthranilic acid (25 μ M) or shikimic acid (25 μ M), which are precursors of tryptophan in eubacteria, resulted in no growth of *M. mobile*. Tryptophan can be converted to indole and skatole by rumen bacteria (25). Indole (45 μ M) could replace tryptophan for the growth of *M. mobile*, but substitution with skatole (38 μ M) resulted in no growth. After this finding, indole was used in place of tryptophan for the routine culture of *M. mobile*.

Vitamin requirements in the presence of crude mobile factor. *M. mobile* was found to require several of the vitamins in the vitamin solution described previously (24). When *M. mobile* was cultured by using a crude source of mobile factor prepared from a boiled cell extract of *Methanobacterium thermoautotrophicum*, as described above, requirements were determined for pyridoxine (0.49 μ M) and thiamine (0.15 μ M) (Table 2). Growth was found to be stimulated by PABA (0.18 μ M), but *M. mobile* could be transferred several times (greater than a 10^6 dilution) in the absence of PABA. Folic acid (0.11 μ M) did not stimulate the growth of *M. mobile*.

Development of a partially defined medium. Culturing of *M. mobile* with a crude preparation of mobile factor was notably improved by the use of a partially defined medium compared with that with the complex medium (Fig. 1). The partially defined medium consisted of the following additions, per liter, to the basal medium described above: sodium acetate, 2.5 g; isobutyric acid, 0.2 g; isovaleric acid, 0.2 g; 2-methylbutyric acid, 0.2 g; indole, 5 mg; pyridoxine hydrochloride, 100 μ g; thiamine hydrochloride, 50 μ g; PABA, 50 μ g; crude mobile factor stock solution, 2 ml. *M. mobile* achieved higher absorbances (here, 0.6 A_{660} versus 0.2 A_{660} for complex medium; Fig. 1), and lysis of the cultures was inhibited (Fig. 1). *M. mobile* could be cultured in the absence of yeast extract and vitamin-free Casamino Acids, but growth was found to be more reproducible from tube to tube in the presence of yeast extract (1 g/liter) or vitamin-free Casamino Acids (2 g/liter) (data not shown).

Vitamin requirements in the presence of purified mobile factor. Crude mobile factor was further purified by anoxic chromatography, as described above, to yield purified mo-

TABLE 2. Effect of vitamins on the growth of *Methanomicrobium mobile* with crude mobile factor

Growth condition ^a	Growth (A ₆₆₀) ^b
Vitamins, complete ^c	0.31
Vitamins — biotin	0.42
Vitamins — pyridoxine	-0.01
Vitamins — thiamine	0.04
Vitamins — vitamin B ₁₂	0.33
Vitamins — PABA	0.19
Vitamins — lipoic acid	0.40
No addition	0.02

^a The basal medium consisted of a mineral medium supplemented with vitamin-free Casamino Acids (2 g/liter); indole (5 mg/liter); acetate, isobutyrate, isovalerate, and 2-methylbutyrate (Table 1); and crude mobile factor (10 µl/5 ml of medium).

^b Growth (after 4 days of incubation) was monitored by the method described by Balch and Wolfe (3).

^c Vitamin mixture was that described by Wolin et al. (24). Final concentrations of vitamins in the medium, in micrograms per liter, were as follow: pyridoxine hydrochloride, 100; thiamine hydrochloride, 50; PABA, 50; nicotinic acid, 50; lipoic acid, 50; riboflavin, 50; calcium pantothenate, 50; biotin, 20; folic acid, 20; vitamin B₁₂, 1.

bile factor. After this purification, the growth of *M. mobile* on partially defined medium was found to be problematic and, again, dependent on the presence of yeast extract (2 g/liter). It was determined that *M. mobile* required biotin (0.04 µM) and vitamin B₁₂ (0.04 µM) for growth with purified mobile factor (Table 3). Biotin and corrinoids were apparently present in the crude mobile factor prepared from boiled cell extract of *Methanobacterium thermoautotrophicum*; preparations of crude mobile factor were usually pink from the presence of corrinoids.

Development of a defined medium. A defined medium was developed for *Methanomicrobium mobile* based on determined optimal levels of volatile fatty acids and vitamins in the presence of purified mobile factor. The defined medium consisted of the following additions, per liter, to the basal medium described above: sodium acetate, 2.5 g; isobutyric acid, 0.057 g; isovaleric acid, 0.075 g; 2-methylbutyric acid, 0.15 g; indole, 5 mg; pyridoxine hydrochloride, 100 µg; thiamine hydrochloride 50 µg; PABA, 25 µg; biotin, 10 µg; vitamin B₁₂, 50 µg; purified mobile factor stock solution, 4 ml. *M. mobile* was maintained by use of this medium.

Requirement for mobile factor. *M. mobile* required a heat-stable factor for growth that is present in rumen fluid (2, 17) and boiled cell extract of *Methanobacterium thermoautotrophicum*. The level of mobile factor in a sample could be estimated by using the defined medium described above. The turbidity of a culture of *M. mobile* increased linearly with the following increasing additions of mobile factor (values are crude mobile factor stock solution added per 5 ml of medium, A₆₆₀ after 5 days of incubation): 0 µl, 0.01; 1 µl, 0.22; 2 µl, 0.31; 4 µl, 0.53; 6 µl, 0.67; 10 µl, 0.70. A bioassay for mobile factor has been established.

DISCUSSION

The erratic growth and lysis of *Methanomicrobium mobile* in the complex medium described by Balch et al. (2) made necessary the development of a medium which could be used for the bioassay of mobile factor. Spontaneous lysis has been described in other methanogens, including *Methanobacterium bryantii* (10), *Methanococcus jannaschii* (11), and *Methanobacterium thermoacaliphilum* (4). However, the rapidity and extent of lysis in cultures of *Methanomicrobium*

mobile in complex medium was especially problematic; not only was it difficult to assay for the unknown growth factor but it also proved challenging just to maintain the organism in the laboratory. There were indications that lysis was due to imbalanced growth or problems with nutrition. Lysis in other methanogens has been associated with poor growth conditions (4, 10). Rapid lysis had been noted during nutrition studies with strains of another ruminal microorganism, that in the genus *Ruminococcus* (6).

A high level of acetate (30 mM) was required by *Methanomicrobium mobile* for growth. *Methanobrevibacter ruminantium*, another rumen methanogen, requires a similar level of acetate (8). Acetate is required at lower concentrations by several other methanogens, including *Methanobrevibacter smithii*, *Methanococcus voltae*, *Methanomicrobium paynteri*, *Methanogenium cariaci*, *Methanogenium olentangyi*, *Methanogenium tatii*, and *Methanoplanus limicola* (22, 26). It has been shown in *Methanobrevibacter ruminantium* (8) and *Methanococcus voltae* (23) that acetate is incorporated into cellular carbon. Acetate is found in many anaerobic environments, including the rumen, from which *Methanomicrobium mobile* was isolated. Acetate has also been found to be important for the growth of many other ruminal bacteria (7).

Methanomicrobium mobile requires the branched-chain fatty acids isobutyrate, isovalerate, and 2-methylbutyrate. *Methanobrevibacter ruminantium* requires 2-methylbutyrate, which is used for isoleucine biosynthesis (8). *Methanococcus voltae* requires isovalerate and 2-methylbutyrate for growth, which are used for leucine and isoleucine biosynthesis, respectively (23). However, unlike the two rumen methanogens, the branched-chain amino acids can substitute for the branched-chain fatty acids in *Methanococcus voltae* (23). Many rumen bacteria require branched-chain fatty acids, and, as with *Methanomicrobium mobile* and *Methanobrevibacter ruminantium*, the branched-chain amino acids do not substitute for the branched-chain fatty acids (5-7, 19).

Methanomicrobium mobile requires the vitamins pyridoxine, thiamine, biotin, and vitamin B₁₂; and growth is stimulated by PABA. Little is known about the exact effect of vitamins on methanogens, although many species require or are stimulated by vitamin solutions (18, 22). Specifically, pantothenate stimulates the growth of *Methanococcus voltae* (23), *Methanococcoides methylutens* requires biotin (20), and *Methanosarcina* sp. strain TM-1 requires PABA for rapid growth (14). In both *Methanomicrobium mobile* and *Methanosarcina* sp. strain TM-1, folic acid does not substitute for the activity of PABA (14). These vitamin requirements indicate that biotin, thiamine, pantothenic acid, pyridoxine, PABA, and vitamin B₁₂ play some role in the metabolism of methanogens. The level of vitamin B₁₂ re-

TABLE 3. Biotin and vitamin B₁₂ requirement of *Methanomicrobium mobile* with purified mobile factor

Addition (per liter) ^a	Growth (A ₆₆₀) ^b
No addition	0.03
Biotin, 50 µg	0.02
Vitamin B ₁₂ 100 µg	0.08
Biotin + vitamin B ₁₂	0.32

^a The basal medium consisted of a mineral medium supplemented with vitamin-free Casamino Acids (2 g/liter); indole (5 mg/liter); acetate, isobutyrate, isovalerate, and 2-methylbutyrate (Table 1); pyridoxine, thiamine, and PABA (Table 2); and purified mobile factor (20 µl/5 ml of medium).

^b Growth (after 6 days of incubation) was monitored by the method described by Balch and Wolfe (3).

quired by *Methanomicrobium mobile* (37 nmol/liter) is in line with the level of corrinoids found in methanogens (0.66 nmol/mg of cell dry weight of *Methanobacterium thermoautotrophicum*) (12). A role in the metabolism of methanogens is also indicated for riboflavin and nicotinic acid, which have been found in significant levels in extracts of *Methanococcus voltae* and *Methanobacterium thermoautotrophicum* (13).

The nutrition of *Methanomicrobium mobile*, which is complex compared with that of most methanogens, is not unusual for a microorganism found in the ruminal environment. Nutritional traits that *Methanomicrobium mobile* shares with many rumen bacteria include requirement for acetate (7); requirement for branched-chain volatile fatty acids and the inability of branched-chain amino acids to substitute for branched-chain volatile fatty acids (5–7, 19); vitamin requirements, including biotin, thiamine, pyridoxine, and vitamin B₁₂ (5–7, 19); and stimulation by PABA (5, 6). With this knowledge of the nutrition of *Methanomicrobium mobile*, it is likely that additional strains of *Methanomicrobium mobile* and other methanogens may be isolated from the rumen and other anaerobic environments.

A defined medium that results in reproducible and stable growth of *Methanomicrobium mobile* has been developed. This will augment research for the isolation and characterization of mobile factor, a novel compound that is required for the growth of *Methanomicrobium mobile*.

ACKNOWLEDGMENTS

We thank William E. Balch for enthusiasm, encouragement, and helpful discussion during the initial phase of this research.

This study was supported by Public Health Service grant AI 12277 from the National Institutes of Health and by grant PCM81-18178 from the National Science Foundation. R.S.T. was the recipient of traineeship GM 7283 from the U.S. Public Health Service.

LITERATURE CITED

- Allison, M. J. 1978. Production of branched-chain volatile fatty acids by certain anaerobic bacteria. *Appl. Environ. Microbiol.* **35**:872–877.
- Balch, W. E., L. J. Magrum, G. E. Fox, R. S. Wolfe, and C. R. Woese. 1979. Methanogens: reevaluation of a unique biological group. *Microbiol. Rev.* **43**:260–296.
- Balch, W. E., and R. S. Wolfe. 1976. New approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere. *Appl. Environ. Microbiol.* **32**:781–791.
- Blotvogel, K.-H., U. Fischer, M. Mocha, and S. Jannsen. 1985. *Methanobacterium thermoalkalophilum* spec. nov., a new moderately alkaliphilic and thermophilic autotrophic methanogen. *Arch. Microbiol.* **142**:211–217.
- Bryant, M. P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. *Fed. Proc.* **32**:1809–1813.
- Bryant, M. P., and I. M. Robinson. 1961. Some nutritional requirements of the genus *Ruminococcus*. *Appl. Microbiol.* **9**:91–95.
- Bryant, M. P., and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. *J. Bacteriol.* **84**:605–614.
- Bryant, M. P., S. F. Tzeng, I. M. Robinson, and A. E. Joyner. 1971. Nutrient requirements of methanogenic bacteria, p. 23–40. In F. G. Pohland (ed.), *Anaerobic biological treatment processes*. Advances in chemistry, series 105. American Chemical Society, Washington, D.C.
- Conway de Macario, E., A. J. L. Macario, and M. J. Wolin. 1982. Antigenic analysis of *Methanomicrobiales* and *Methanobrevibacter arboriphilus*. *J. Bacteriol.* **152**:762–764.
- Jarrell, K. F., J. R. Colvin, and G. D. Sprott. 1982. Spontaneous protoplast formation in *Methanobacterium bryantii*. *J. Bacteriol.* **149**:346–353.
- Jones, W. J., J. A. Leigh, F. Mayer, C. R. Woese, and R. S. Wolfe. 1983. *Methanococcus jannaschii* sp. nov., an extremely thermophilic methanogen from a submarine hydrothermal vent. *Arch. Microbiol.* **136**:254–261.
- Krzycki, J., and J. G. Zeikus. 1980. Quantification of corrinoids in methanogenic bacteria. *Curr. Microbiol.* **3**:243–245.
- Leigh, J. A. 1983. Levels of water-soluble vitamins in methanogenic and non-methanogenic bacteria. *Appl. Environ. Microbiol.* **45**:800–803.
- Murray, P. A., and S. H. Zinder. 1985. Nutritional requirements of *Methanosarcina* sp. strain TM-1. *Appl. Environ. Microbiol.* **50**:49–55.
- Nolan, R. A. 1971. Amino acids and growth factors in vitamin-free casamino acids. *Mycologia* **63**:1231–1234.
- Noll, K. M., K. L. Rinehart, Jr., R. S. Tanner, and R. S. Wolfe. 1986. Structure of component B (7-mercaptoheptanoylthreonine phosphate) of the methylcoenzyme M methylreductase system of *Methanobacterium thermoautotrophicum*. *Proc. Natl. Acad. Sci. USA* **83**:4238–4242.
- Paynter, M. J. B., and R. E. Hungate. 1968. Characterization of *Methanomicrobium mobilis*, sp. n., isolated from the bovine rumen. *J. Bacteriol.* **95**:1943–1951.
- Rivard, C. J., and P. H. Smith. 1982. Isolation and characterization of a thermophilic marine methanogenic bacterium, *Methanogenium thermophilicum* sp. nov. *Int. J. Syst. Bacteriol.* **32**:430–436.
- Slyter, L. L., and J. M. Weaver. 1977. Tetrahydrofolate and other growth requirements of certain strains of *Ruminococcus flavefaciens*. *Appl. Environ. Microbiol.* **33**:363–369.
- Sowers, K. R., and J. G. Ferry. 1985. Trace metal and vitamin requirements of *Methanococcoides methylutens* grown with trimethylamine. *Arch. Microbiol.* **142**:148–151.
- Weisburg, W. G., and R. S. Tanner. 1982. Aminoglycoside sensitivity of archaeobacteria. *FEMS Microbiol. Lett.* **14**:307–310.
- Whitman, W. B. 1985. Methanogenic bacteria, p. 3–84. In C. R. Woese and R. S. Wolfe (ed.), *Archaeobacteria. The bacteria*, vol. VIII. Academic Press, Inc., New York.
- Whitman, W. B., E. Ankwarda, and R. S. Wolfe. 1982. Nutrition and carbon metabolism of *Methanococcus voltae*. *J. Bacteriol.* **149**:852–863.
- Wolin, E. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. *J. Biol. Chem.* **238**:2882–2886.
- Yokoyama, M. T., and J. R. Carlson. 1974. Dissimilation of tryptophan and related indolic compounds by ruminal microorganisms in vitro. *Appl. Environ. Microbiol.* **27**:540–548.
- Zabel, H. P., H. König, and J. Winter. 1984. Isolation and characterization of a new coccoid methanogen, *Methanogenium tatii* spec. nov. from a sulfataric field on Mount Tatío. *Arch. Microbiol.* **137**:308–315.