APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Feb. 1989, p. 514–515
0099-2240/89/020514-02$02.00/0

Vol. 55, No. 2

Threshold Acetate Concentrations for Acetate Catabolism by Aceticlastic Methanogenic Bacteria

PETER WESTERMANN,† BIRGITTE K. AHRING,‡ AND ROBERT A. MAH*

Division of Environmental and Occupational Health Sciences, School of Public Health, University of California, Los Angeles, California 90024

Received 9 August 1988/Accepted 15 November 1988

Marked differences were found for minimum threshold concentrations of acetate catabolism by *Methanosarcina barkeri* 227 (1.180 mM), *Methanosarcina mazei* S-6 (0.396 mM), and a *Methanothrix* sp. (0.069 mM). This indicates that the aceticlastic methanogens responsible for the conversion of acetate to methane in various ecosystems might be different, depending on the prevailing in situ acetate concentrations.

Acetate is one of the most important intermediates during degradation of organic matter in anaerobic environments (2, 10). Under methanogenic conditions, i.e., when inorganic electron acceptors such as sulfate, ferric iron, or nitrate are absent, acetate is degraded to CH_4 and CO_2 by *Methanothrix* and *Methanosarcina* species, the only known methanogens capable of acetate catabolism (8, 12).

Several authors reported that pronounced differences exist for the minimum, or threshold, concentration of hydrogen which can be metabolized by various anaerobic hydrogen-utilizing bacteria (3, 6). In competitive interactions among different species of bacteria, the capability for growth on the lowest concentration of a growth-limiting substrate may be the major factor determining the population outcome under steady-state conditions (3, 6).

This threshold model was applied mainly to competing populations of different physiological groups of bacteria (sulfate reducers and methanogens). In this system, the sulfate-reducing bacteria outcompete methanogenic bacteria by maintaining the hydrogen partial pressure below the minimum threshold necessary for methane production (6). Within these bacterial groups, however, significant differences in threshold values for common substrates might exist, and competition can occur as well among species of the same genus or physiological group.

Large variations occur in the acetate concentrations of different methanogenic environments. Consequently, different acetate-decarboxylating methanogens may be favored if they have different threshold values for acetate utilization. The aim of the present study was to examine whether such differences exist among three strains of acetate-utilizing methanogenic bacteria.

Methanosarcina barkeri 227 and *Methanosarcina mazei* S-6 came from our culture collection and were grown in a medium with the following composition (grams per liter): NH_4Cl, 1.0; $K_2HPO_4 \cdot 3H_2O$, 0.4; $MgCl_2 \cdot 6H_2O$, 1.0; $CaCl_2 \cdot 2H_2O$, 0.5; cysteine hydrochloride, 0.5; $NaHCO_3$, 3.8; $Na_2S \cdot 9H_2O$, 0.25; Trypticase peptone, 2.0; yeast extract, 2.0; resazurin, 0.001. In addition, 10 ml of a trace metal solution (4) was added per liter. A highly purified culture of a *Methanothrix* sp. enriched from anoxic mud from the Los Angeles River was grown in the same medium, with acetate as the sole carbon and energy source but without Trypticase peptone and yeast extract. All experiments were carried out in 50-ml serum vials (20 ml of medium per vial) closed with butyl rubber stoppers and aluminum crimps under a 70:30 N_2-CO_2 atmosphere. Acetate was added to the vials from sterile stock solutions stored under N_2. All incubations were carried out at 37°C under static conditions. Acetate was measured by capillary gas chromatography with a Hewlett Packard 5890 equipped with the following column: 10 m by 0.53 mm, Superox, 1.2. μm (Alltech Associates, Inc.). The oven temperature was 105°C, and the injector and flame ionization detector temperature was 180°C. Samples were acidified to a pH of <2 with H_3PO_4, and 0.5 μl of each sample was analyzed.

Representative curves for depletion of acetate by the three methanogenic bacteria are shown in Fig. 1, and acetate thresholds when no further depletion occurred (incubation of more than 1 month) are shown in Table 1. Our results show a clear difference between threshold values of the three methanogens. The *Methanothrix* sp. had a threshold value less than 6% that of *M. barkeri* and less than 18% that of *M. mazei*, while the threshold value of *M. mazei* was less than 35% that of *M. barkeri*. When 10 mM acetate was added to stationary-phase cultures, similar threshold values were observed (data not shown).

To our knowledge, the only methanogenic threshold value reported for acetate is 25 to 75 μM in a thermophilic, butyrate-degrading triculture in which the reported acetate-decarboxylating methanogen is a rod-shaped bacterium (1). These concentrations correspond well with the threshold value for *Methanothrix* sp. found in this study.

As previously mentioned, acetate concentrations vary significantly between different anaerobic environments. In digestors, the acetate concentration varies between 0.3 and 5.8 mM (5, 10), depending largely on the loading rate of organic material. In freshwater sediments and in waterlogged soils, the acetate concentration rarely exceeds a few hundred millimolar (9, 11; manuscript in preparation). If our data are representative for methanogens in their natural environments, differences between in situ concentrations might be reflected in the composition of the aceticlastic population of methanogens. In digestors in which acetate concentrations exceed 1 mM, all three methanogenic species are potentially active in acetate decarboxylation. In well-balanced digestors with acetate concentrations well below 1 mM, *Methanothrix* species and *M. mazei* are expected to be

* Corresponding author.
† Present address: Department of General Microbiology, University of Copenhagen, Solvgade 83H, 1307 Copenhagen K, Denmark.
‡ Present address: Institute of Biotechnology, The Technical University of Denmark, 2800, Lyngby, Denmark.

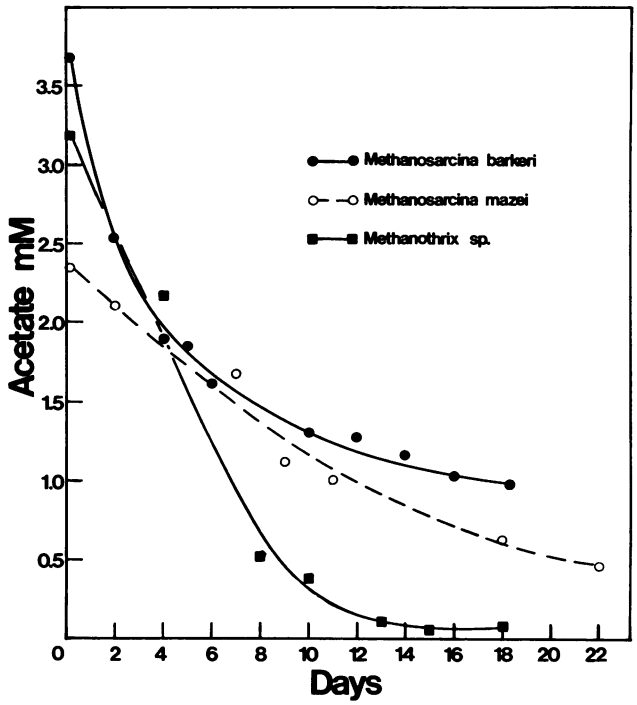

FIG. 1. Catabolism of acetate by *M. barkeri* 227, *M. mazei* S-6, and *Methanothrix* sp. Since the inoculm size was different for each species, depletion rates could not be compared.

the predominant aceticlastic methanogens, while *M. barkeri* will be confined to microniches with higher acetate concentrations. In freshwater sediments and waterlogged soils with acetate concentrations below 400 μM, only *Methanothrix* species are able to produce methane from acetate. Under these conditions, *Methanosarcina* species may utilize only substrates such as methanol and methylamines.

Macario and Conway de Macario (7) developed immunoprobes to test the in situ population composition of methanogenic bacteria. A more widespread analysis of methanogenic ecosystems by this or related methods together with chemical characterizations of the environments is necessary to verify the effect of the threshold patterns observed in this study.

TABLE 1. Threshold values for acetate metabolism

Bacterium	Threshold (mM) ± SD	*n*
M. barkeri	1.180 ± 0.259	11
M. mazei	0.397 ± 0.116	6
Methanothrix sp.	0.069 ± 0.034	6

This study was supported by the Danish Natural Research Council (grant 11-6577), a NATO fellowship, and the Nordic Ministerial Council. It was also sponsored by a collaborative program (Methane from Biomass and Wastes) of the Institute of Food and Agricultural Sciences of the University of Florida and the Gas Research Institute.

LITERATURE CITED

1. **Ahring, B. K., and P. Westermann.** 1987. Kinetics of butyrate, acetate, and hydrogen metabolism in a thermophilic, anaerobic, butyrate-degrading triculture. Appl. Environ. Microbiol. **53:** 434–439.
2. **Cappenberg, T. E., and R. A. Prins.** 1974. Interrelations between sulfate-reducing and methane-producing bacteria in bottom deposits of a freshwater lake. III. Experiments with ^{14}C-labelled substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **40:**457–469.
3. **Cord-Ruwisch, R., H.-J. Seitz, and R. Conrad.** 1988. The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. Arch. Microbiol. **149:**350–357.
4. **Ferguson, T. J., and R. A. Mah.** 1983. Isolation and characterization of an H_2-oxidizing thermophilic methanogen. Appl. Environ. Microbiol. **45:**265–274.
5. **Kaspar, H. F., and K. Wuhrmann.** 1978. Kinetic parameters and relative turnovers of some important catabolic reactions in digesting sludge. Appl. Environ. Microbiol. **36:**1–7.
6. **Lovley, D. R.** 1985. Minimum threshold for hydrogen metabolism in methanogenic bacteria. Appl. Environ. Microbiol. **49:** 1530–1531.
7. **Macario, A. J. L., and E. Conway de Macario.** 1988. Quantitative immunologic analysis of the methanogenic flora of digestors reveals a considerable diversity. Appl. Environ. Microbiol. **54:**79–86.
8. **Mah, R. A., M. R. Smith, and L. Baresi.** 1978. Studies on an acetate-fermenting strain of *Methanosarcina*. Appl. Environ. Microbiol. **35:**1174–1184.
9. **Molongoski, J. J., and M. J. Klug.** 1983. Anaerobic metabolism of particulate organic matter in the sediments of a hypereutrophic lake. Freshwater Biol. **10:**507–518.
10. **Smith, P. H., and R. A. Mah.** 1966. Kinetics of acetate metabolism during sludge digestion. Appl. Microbiol. **14:**368–371.
11. **Strayer, R. F., and J. M. Tiedje.** 1978. Kinetic parameters of the conversion of methane precursors to methane in a hypereutrophic lake sediment. Appl. Environ. Microbiol. **36:**330–340.
12. **Zehnder, A. J. B., B. A. Huser, T. D. Brock, and K. Wuhrmann.** 1980. Characterization of an acetate-decarboxylating, non-hydrogen-oxidizing methane bacterium. Arch. Microbiol. **149:** 350–357.