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Chemotaxis in *Methanospirillum hungatei*

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***Methanospirillum hungatei* gave a positive chemotactic response to acetate.**

Tactic responses by members of the Archaebacteria have received limited attention. *Halobacterium halobium* exhibits phototaxis and chemotaxis (9, 11), but there is only one brief report on chemotactic responses in a methanogen, *Methanococcus voltae* (K. A. Sment and J. Konisky, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, I29, p. 185).

Methanospirillum hungatei is a methanogen that grows on CO_2-H_2 or formate. Acetate can be utilized as a carbon source (6). Cells are curved, 0.5 by 7 μm in diameter, often forming long spiral filaments. The outermost cell layer is rigid, composed of protein subunits, and extremely resistant to disruption (4, 10). The ends of the filaments, through which tufts of flagella are inserted, are composed of several layers of material which is different from the sheath (10).

Cells of methanospirillum may have mono- or bipolar flagella. This is similar to the genus *Halobacterium* (2) and different from most polarly flagellated eubacteria. Motile cells swim smoothly in one direction, often for as long as several minutes, with the cell cylinder rotating. Negatively stained preparations often contain cells with the tuft of flagella at one end of the cell extended, while that at the other end is bent along the cell cylinder. There is no motion between smooth runs comparable to the tumbles of *Escherichia coli* and *Salmonella* sp. (3, 8) or the flexing of spirochetes (7). In general, longer filaments move more slowly than short filaments, but cell velocity of even a single cell is quite variable with time (D. L. Cruden, R. Sparling, and A. J. Markovetz, submitted for publication). The present study was undertaken to determine whether *M. hungatei* shows chemotactic responses.

Cells of *M. hungatei* GP1 were grown in the medium of Daniels et al. (5) in serum-stoppered tubes under ~200 pK_a H_2 + CO_2 (80%-20%). For testing chemotaxis in response to formate, cells were grown in the same medium with the addition of 200 mM sodium formate, and tubes were gassed with N_2-CO_2. After growth to a density of about 1.7×10^7 cells ml^{-1}, cells were washed twice anaerobically by centrifugation (20 min at 7,000 × *g*) and suspended in anaerobic salts solution, which is the growth medium without acetate or vitamins.

A capillary assay was adapted from Adler (1) for use in the anaerobic chamber (Coy Manufacturing). Bacteria suspended in the anaerobic salts solution to a concentration of 1.6×10^6 cells ml^{-1} were placed in chemotaxis chambers consisting of a microscopic slide, a U-shaped bent capillary, and a square cover slip fastened together with high-vacuum grease. The free end of a capillary tube containing 5 μl of the attractant to be tested and sealed at the other end with vacuum grease was inserted beneath the cover slip into the suspension of bacteria in the pond. At the end of the incubation period, the capillary tube was removed and the free end was sealed with plasticine before being removed from the anaerobic chamber. The contents of each capillary were blown into a Petroff-Hausser chamber and counted. It was assumed that the contents of the capillary were uniformly mixed on the Petroff-Hausser slide. Therefore, the concentration of cells and total number of cells which had migrated into each capillary could be calculated.

All glassware was preincubated in the anaerobic chamber to remove adsorbed O_2, and all solutions were sterile and anaerobic. Twelve assays were performed at a time, including duplicates of each concentration of substrate. Control experiments showed that 45 min at the anaerobic chamber temperature of 25°C gave optimal and reproducible accumulation of cells and that cells remained actively motile for this length of time under the assay conditions.

Figure 1 shows the results from a typical experiment with sodium acetate. The observed threshold concentration, i.e., the amount of attractant giving a response greater than that in the absence of the attractant, was 0.1 mM, and the maximal response occurred at 20 mM acetate. There was no chemotactic response to sodium formate, whether the cells were grown on formate or H_2-CO_2.

To determine whether the response to increasing concen-

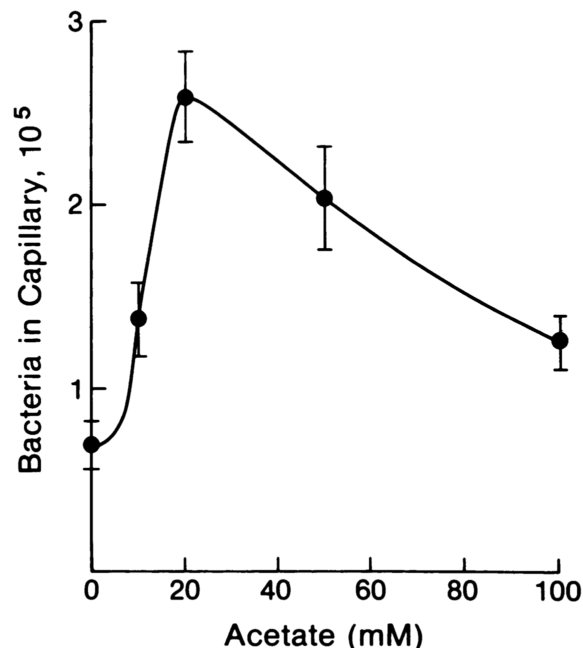

FIG. 1. Chemotactic response of *M. hungatei* to increasing concentrations of acetate. Each point is the mean of the numbers of cells that accumulated in duplicate capillary tubes.

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TABLE 1. Effect of addition of acetate to pond on accumulation of *M. hungatei* in capillaries

Pond content(s)	Capillary content(s)	No. (10^5) of bacteria in capillary[a]
Salts	Salts	1.02
Salts	Salts–10 mM acetate	2.33
Salts	Salts–20 mM acetate	2.80
Salts–10 mM acetate	Salts–10 mM acetate	1.1
Salts–10 mM acetate	Salts–20 mM acetate	2.3

[a] Means of duplicate capillaries in a single experiment are shown (accumulation after 45 min).

trations of acetate in the capillaries was due simply to an increase in motility in the presence of acetate, control experiments were run in which acetate was present in the pond as well as the capillaries. Data from one such experiment are shown in Table 1. The number of cells that accumulated in capillary tubes containing 10 mM acetate from a pond containing 10 mM acetate was almost identical to that in the control capillaries containing salts from a pond containing salts. The accumulation in capillaries containing 20 mM acetate from a pond with 10 mM acetate was similar to that in the capillary with 10 mM acetate from a salts pond. Thus, the response appears to be caused by the difference in concentrations of acetate rather than simply the presence of acetate. This is characteristic of bacterial chemotaxis. Such a response with this methanogen is weaker than the chemotactic responses of enteric bacteria.

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