Chemotaxis in the Archaebacterium Methanococcus voltae

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Received 17 October 1988/Accepted 25 January 1989

The archaebacterium Methanococcus voltae, was shown to be chemotactic. Acetate, isoleucine, and leucine were identified as attractants; whereas histidine was not an attractant. A motile, generally nonchemotactic mutant was isolated.

Tactic responses have been observed in a wide range of organisms in all three of the urkingdoms: the eucaryotes (4, 13), eubacteria (2, 6, 7, 12), and archaebacteria (10). The archaebacteria fall into two major divisions (15). Division I comprises the sulfur-dependent thermophlic archaebacteria. Division II consists of the three major methanogen families, extreme halophiles, and two types of thermoacidophiles. The family Methanococcales is separated from the other methanogens and relatives by the deepest degree in that grouping. It was therefore of interest whether Methano
coccus voltae, a member of the Methanococcales and thus distantly related to the chemotactic halophile Halobacterium halobium, shows chemotactic behavior. Since M. voltae is motile and transports amino acids (5), it seemed plausible that M. voltae would be able to respond to its environment. Here we report evidence of chemotaxis in M. voltae and the isolation of a mutant defective in general chemotaxis.

In addition to H₂ and CO₂, M. voltae requires acetate, isoleucine, and leucine for growth (11, 14). Although it has been shown that neither metabolism nor transport is required for sensing an attractant in Escherichia coli (8), these three compounds were reasonable substances to test as attractants. To determine if M. voltae was chemotactic toward any of these compounds, cells were spotted on acetate, leucine, or isoleucine swarm agar gradient plates. Gradient swarm agar plates were made by pouring 10 ml of swarm agar (0.35%) in defined medium (14) containing 10 mM sodium acetate, 7.5 mM l-isoleucine, and 7.5 mM l-leucine into a petri dish and allowing the agar to solidify on an angle. Ten ml of defined swarm agar lacking acetate, isoleucine, or leucine was then added to leveled plates. Approximately 10⁷ washed cells in 10 μl of 0.4 M NaCl were spotted on the side of the plate with the lowest concentration of solute. Unless otherwise stated, all plating and assay manipulations were performed in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Mich.) with anaerobic solutions. The plates were incubated in brass and stainless steel cannisters under a H₂-CO₂ (80:20) atmosphere to which H₂S was added to a final concentration of 0.2%. Over a 1-week period, M. voltae moved toward the higher concentrations of acetate and leucine but not isoleucine. However, isoleucine was later identified as an attractant with a higher threshold concentration in the capillary tube assays. M. voltae may have not responded to the isoleucine gradient in swarm agar plates because the cells were not placed on the plate at the threshold concentration.

Chemotaxis assays were performed by the method of Adler (1) with minor modifications. Methanogen cultures were grown at 30°C in defined medium under a pressurized atmosphere of H₂-CO₂ to an optical density at 660 nm of approximately 0.6. The cells were centrifuged, gently washed, and suspended in chemotaxis buffer (10 mM potassium phosphate buffer, pH 7.0 [pH 6.5 under 20% CO₂], 0.4 M NaCl, 1 mM dithiothreitol) to a final cell concentration of approximately 5 × 10⁷/ml. Chemotaxis chambers were filled with 0.2 ml of the above methanogen suspension. Capillary tubes (1-μl Micro Pipets; Curtin Matheson Scientific, Inc., Houston, Tex.) were sealed at one end in a flame outside the anaerobic chamber and were filled with the attractant solution in the chamber as follows. The capillary tubes were placed open end down in a small vial containing 5 ml of the attractant in chemotaxis buffer. The open vial was placed in the anaerobic chamber interlock. The interlock atmosphere was placed under a vacuum of −12 lb/in² (~40 kPa) and immediately brought back to atmospheric pressure. About 1.0 cm of the capillaries was filled with solution. The chemotaxis assays were performed in an anaerobic glove box under a N₂-CO₂-H₂ (76:19:5) atmosphere. The capillary was inserted open end first into the chamber containing the methanogen suspension. The tubes were incubated for 30 min at 25°C unless otherwise mentioned. The temperature was controlled by using a Multi-Blok Heater (Lab-Line Instruments, Inc., Melrose Park, Ill.). The capillary was removed at the end of the assay and rinsed with chemotaxis buffer. The sealed end was broken off and the contents were poured into a tube containing 0.1 ml of chemotaxis buffer. This suspension was mixed with 20 ml of molten defined agar containing 1.8% agar medium, poured into a petri dish, and allowed to solidify. After incubation for 7 to 10 days at 37°C, colonies were counted. All assay points were performed in triplicate. Since cultures of M. voltae are prone to autoysis, plate counts of the washed methanogens were routinely determined to ensure lack of significant lysis which could affect results. The terms used in describing the results of the chemotaxis assays have been previously defined by Mesibov and Adler (7).

M. voltae PS was chemotactic toward acetate, leucine, and isoleucine in capillary assays. The rates of accumulation of the methanogens in capillaries containing various concentrations of acetate are shown in Fig. 1. When the capillary contained no attractant (bottom curve), few cells entered the capillary. Chemotaxis was observed with 10 and 100 mM acetate, with more cells entering the capillary with 100 mM acetate. This capillary assay was performed at 30°C for up to 40 min. Longer incubation times in the anaerobic glove box resulted in evaporation of the buffer in the chemotactic chambers. Later assays were performed at 25°C to further decrease the rate of evaporation which caused erratic results.

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This concentration of PS-3 out at carried M. (wt) histidine toward isoleucine concentrations concentration peak cine, and always was observed for isoleucine concentrations. The assays were carried out at 30°C.

in the number of cells entering the capillary. This drop in temperature from 30 to 25°C led to an approximate sixfold decrease in chemotaxis (data not shown). Temperature dependence of chemotaxis over this temperature range has been observed in E. coli (1) and Spirochaeta aurantium (6) but not in Bacillus subtilis (9).

Figures 2, 3, and 4 show the 30-min concentration-response curves of wild-type M. voltae PS to acetate, isoleucine, and leucine, respectively. In contrast the number of cells which moved into the capillary containing buffer alone was always less than 10 under these assay conditions. The peak concentration for acetate was 100 mM. The peak concentrations for isoleucine and leucine could not be determined since both are insoluble above 100 mM in the chemotaxis buffer. M. voltae showed no chemotactic behavior toward acetate, isoleucine, or leucine when an equal concentration of the same compound was present in the cell suspension (data not shown).

Spontaneous mutants of the histidine auxotroph M. voltae PS-3 (3) deficient in chemotaxis were enriched as follows. M. voltae PS-3 was spotted on a swarm agar plate opposite the histidine biosynthetic regulatory mutant M. voltae KS817-5. This regulatory mutant is resistant to the histidine analog 1,2,4-triazole-3-alanine and excretes histidine, proline, tyrosine, and possibly other amino acids or compounds (unpublished results). The histidine necessary for the growth of PS-3 was supplied by KS817-5. M. voltae PS-3 moved from its initial point of inoculation up the concentration gradient formed by KS817-5 over a period of 10 days (Fig. 5). It was not known to what attractant PS-3 responded. This chemotactic behavior toward KS817-5 was not altered by the addition of histidine to the medium, and it was later shown that histidine is not an attractant (see below).

The section of agar was cut out at the point of inoculation, behind the front of cells as they moved up the concentration gradient. This agar section was inoculated into 5 ml of defined medium containing 100 μM histidine and was incubated for 2 days. The culture was diluted to approximately 10⁶ CFU/ml. Molten swarm agar (20 ml) containing histidine was mixed with 0.1 ml of the above dilution and poured into a petri dish. The plates were incubated at 30°C for 3 days. Nonswarming colonies were picked, inoculated into liquid medium, and rechecked for the inability to swarm. A total of 22 nonswarming colonies were isolated out of approximately 1,000 colonies plated. One isolate, Che6, maintained its nonswarming phenotype and was kept for further study.

The concentration-response curves of M. voltae PS-3 and the chemotactic mutant Che6 are also shown in Fig. 2, 3, and 4. PS-3, the parent strain of Che6, was chemotactic toward acetate, isoleucine, and leucine. However, the number of
these colonies did not swarm as far as the wild type or PS-3. Second, the cells of the chemotactic mutant were motile as judged by phase microscopy. Therefore, the inability of these mutants to respond to acetate, isoleucine, and leucine gradients is due to a general defect in the chemotactic machinery.

This work was supported by Department of Energy research grant DEFG 0284 ER 132 and National Science Foundation grant BBS-8714603.

LITERATURE CITED