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Chemotaxis in *Bdellovibrio bacteriovorus*

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Chemotaxis toward yeast extract is demonstrated in obligately and facultatively parasitic strains of *Bdellovibrio bacteriovorus*.

Chemotaxis is the movement of a cell or multicellular organism toward or away from a chemical. It is a fundamental way in which cells at all levels of biological organization may sense and respond to their physical and biological environments. In microorganisms, chemotaxis is a means of coordinating cellular aggregation (8), of locating favorable concentrations of oxygen and nutrients (1, 16), and of avoiding toxic conditions (15–17). In predacious bacteria (9) and fungi (6), chemotaxis may function in locating and maintaining proximity to the host organism.

Bdellovibrio bacteriovorus is a parasitic bacterium which can grow and reproduce within the periplasmic space of gram-negative host bacteria. Highly motile bdellovibrio progeny are released upon lysis of the host cells (14). The viability of these liberated cells drops sharply within a few hours after release (11), and during this time period the parasites must successfully locate and enter new host cells. The search for and predation of additional host cells would be greatly facilitated if bdellovibrio could detect them from a distance through a chemotactic response to substances released by the host cells. This would be of considerable value for survival in environments which contain relatively small microbial populations. To date, no studies have been reported which demonstrate whether or not *B. bacteriovorus* possesses the capacity for chemotaxis. Our observations reveal that both obligately and facultatively parasitic strains of *B. bacteriovorus* exhibit chemotaxis.

B. bacteriovorus strains UKi2 (facultatively parasitic) and 114 (obligately parasitic) were grown at 30 C on *Escherichia coli* B in dilute nutrient broth supplemented with Ca^{2+} and Mg^{2+} (13). Host-independent cultures of strain UKi2 were grown in peptone-yeast extract (Difco) broth (7). Before use in a chemotaxis experiment, the bdellovibrios were given three 12-h transfers using 10% (vol/vol) inocula.

The resulting cultures of *B. bacteriovorus* were filtered to remove most of the remaining host cells (in lysates) or long spirals (for host-independently grown cultures); then they were washed once with, and resuspended in, distilled water containing 0.001 M tricine (*N*-tris(hydroxymethyl)methylglycine·NaOH), pH 7.35, 0.002 M $CaCl_2$, and 0.003 M $MgCl_2$. The densities of the suspensions of bdellovibrio were adjusted by dilution after enumeration with a Petroff-Hausser counting chamber (10). The motility of the parasites was unimpaired by the harvesting process and by incubation for up to 4 h in the suspending medium.

The attractant solution of 0.5% (wt/vol) yeast extract (Difco) was made in suspending medium, the pH was adjusted to 7.35 with NaOH, and the solution was sterilized by filtration. All dilutions of this solution were made into suspending medium.

Chemotaxis was measured by the capillary method of Pfeffer as modified by Adler (4). For each attractant or control solution, three capillaries containing the test substance were incubated for 30 min, each with 0.3 ml of the suspension of bdellovibrio containing 10^8 cells/ml. The contents of the capillaries were expelled, each into 10 ml of dilute nutrient broth, and two decimal dilutions of these suspensions were made into dilute nutrient broth. The bdellovibrios in the suspensions were enumerated as plaque-forming units on double-layer plates. These were prepared as previously described (10), except that the medium was dilute nutrient agar.

Figure 1 shows the accumulation of cells of *B. bacteriovorus* strain UKi2 inside a capillary containing 0.5% yeast extract. Accumulation first becomes clearly apparent several minutes after insertion of the capillary into the suspension of cells: a diffuse cloud of cells forms inside the capillary near the opening. This cloud enlarges and migrates down the capillary, leaving a less dense area near the opening (Fig. 1b). A few minutes later, several discrete bands of cells form from the cloud. Figure 1c shows two such bands. Farther down the capillary there was also a third, broad band. The formation of

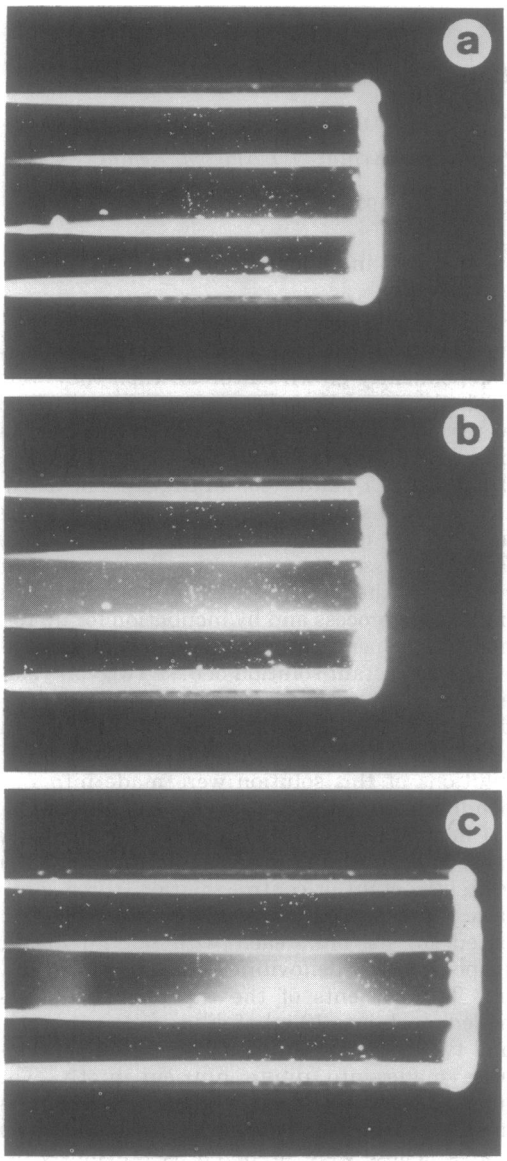

FIG. 1. *Formation of chemotactic bands by B. bacteriovorus strain UKi2 attracted to 0.5% yeast extract in a capillary. (a) 1.0 min after insertion of the capillary into the suspension of bdellovibrio; (b) 10.5 min after insertion; (c) 19.5 min after insertion. The bright specks on the capillary arise from imperfections in the glass. Total magnification, 29×.*

such clouds of cells in response to a gradient of a chemotactic attractant has been used as evidence of chemotaxis in microorganisms ever since the first descriptions of the phenomenon in the late 1800's. The formation of bands inside the capillary could arise from sequential, selective metabolism of substances in the yeast extract. Cells utilizing a given substance would migrate up a gradient created by their own metabolism (1).

Figure 2 shows the responses to several concentrations of yeast extract of *B. bacteriovorus* strain UKi2 grown both host dependently and host independently and of *B. bacteriovorus* strain 114. The three curves show a smooth increase in accumulation of cells in the capillaries as the concentration of yeast extract increases. This probably is due to an increasingly strong chemotaxis of the cells in response to increasing concentrations of attracting compounds in the yeast extract. It is not due to "trapping" of cells in the capillaries resulting from decreased motility in yeast extract: cells in suspending medium could be highly diluted into 0.5% yeast extract at 30 C without any noticeable effect on their motility. The formation and migration of bands of cells in the capillaries (Fig. 1) also supports our belief that the cells are accumulating in the capillaries as a result of chemotactic migration.

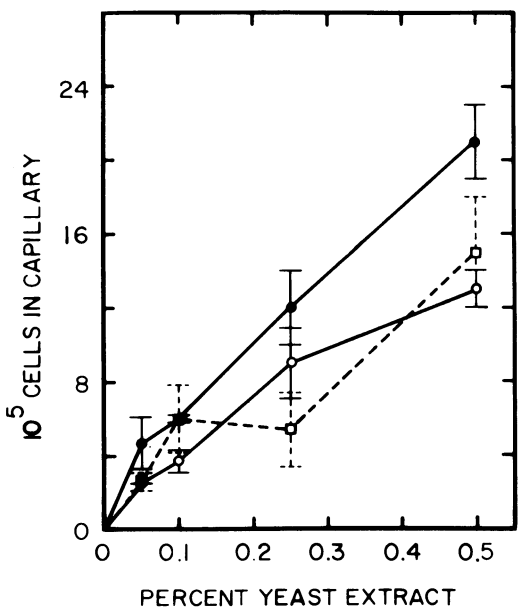

FIG. 2. *Response of B. bacteriovorus strain UKi2 grown both host dependently and host independently and of B. bacteriovorus strain 114 to several concentrations of yeast extract. Symbols:* (●), *strain UKi2 grown host dependently;* (○), *strain UKi2 grown host independently;* (□), *strain 114. The points are the average of three (for* ● *and* □) *or two (for* ○) *replicate determinations. The error bars delineate the range of values* (○) *or* ± *one standard deviation of the values* (● *and* □). *The values have been corrected for the numbers of cells entering capillaries containing the suspending medium. These numbers were: 1.8* ± *0.0* × 10^4 (○), *5.7* ± *0.9* × 10^4 (●), *and 1.2* ± *0.4* × 10^5 (□).

In the experiments of Fig. 2, the different modes of growth of *B. bacteriovorus* strain UKi2 had little effect on the chemotaxis of the resulting cultures. The differences in strengths of chemotactic responses shown by the differently cultured *B. bacteriovorus* strain UKi2 and by strain 114 are probably not of significance, since there is variability in the absolute magnitudes of the responses in replicate experiments done on different days. However, the curves do show strong responses by both strains. This may reflect a metabolic capability common to all bdellovibrios, since the two strains tested here are of independent origin and differ in their degree of host dependency: strain 114 is obligately parasitic, whereas strain UKi2 is facultatively parasitic. (The plating efficiency of strain 114 as plaque-forming units was 100%; efficiency as colony-forming units was 0%. For strain UKi2 the plating efficiency as plaque-forming units was 100%; as colony-forming units it was 50 to 70% in these two experiments.)

The data of Fig. 1 and 2 show that both strains of *B. bacteriovorus* have the capacity for chemotaxis. The formation of three bands of cells in the capillary tubes containing yeast extract implies that *B. bacteriovorus* strain UKi2 is chemotactic to at least three substances, one of which is probably oxygen (1, 2). Studies like those of Adler (1, 2, 4, 11) would show the range of compounds sensed by bdellovibrios and the number of chemoreceptors (3) present.

Some experiments have been performed in which exudates of *E. coli* and *Bacillus cereus* served as sources of "natural" attractants; these preparations elicited only weak chemotactic responses from the bdellovibrios. Several possible explanations for these results are currently being explored.

Clearly, further work is required before the significance of chemotaxis in the parasitic life cycle of *B. bacteriovorus* will be understood. However, the presence of the capacity for chemotaxis implies that it is of adaptive significance.

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