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A modified capillary assay for chemotaxis

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SUMMARY

A modification is described of the capillary assay for chemotaxis. It employs a 96-well dilution plate and its cover. Capillary tubes are inserted through the cover and are supported by small rubber collars. The method is faster and less tedious and gives more precise results than earlier methods.

INTRODUCTION

Motility is one of the most readily demonstrated bacterial characters, and chemotaxis is one of the most easily studied bacterial behaviors. Since bacteria devote about 50 genes, some 3–5% of their genome [10], and their products to motility and chemotaxis, it is widely held that these properties provide a competitive advantage, at least under some environmental conditions. Moreover, chemotaxis is a virulence factor in strains of *Salmonella* and in *Vibrio cholerae* [5,6,12]. Motility and chemotaxis have been the subjects of several reviews [3,7,8,10].

The capillary assay developed by Adler [1,2,4] provided a ready method for quantifying chemotaxis. In this method, a capillary tube containing a potential attractant or repellant is placed in a 'pond' containing a suspension of motile bacteria. The number of bacteria which swim into the tube in a known period is compared with the number which swim into a control tube lacking the compounds. An increased number in the tube compared to the control indicates the compound is an attractant and a decreased number indicates it is a repellant.

In the original assay, the pond with bacteria is contained inside a small U-shaped glass rod on a microscope slide. The U-shaped rod is placed on the slide and a cover slip is placed over it. The suspension of bacteria in medium is allowed to flow into this chamber, forming the pond, and a capillary, closed at one end and containing the same medium and a potential attractant or repellant, is inserted open-end-first into the pond. At the end of the experimental period the cover slip is removed and the capillary is picked up with tweezers, the outside is rinsed with sterile water, the tube is broken aseptically, and a small rubber bulb assembly is attached to it and used to flush the contents of the tube into diluent for further dilution and plating. A plexiglass chamber devised by Palleroni [14] decreased some of the tedium involved and eliminated the tendency of the pond to leak out under the U-tube in mid-assay. Other modifications include using digital image processing to quantify the accumulation of bacteria near the mouth of the capillary instead of counting viable cells in the tube by a plate count [13], and quantifying bacteria in the capillary using a particle counter [15].

In the hands of several workers in our laboratory, the results obtained from replicates within a single experiment and between replicate experiments had unacceptable variability. Colleagues at other institutions indicated that they had similar difficulties. This paper presents a way to reduce some of that variability and some of the tedium associated with manipulating the capillary.

MATERIALS AND METHODS

Organisms and culture conditions

Serratia sp. Gil-1 and *Pseudomonas fluorescens* SHC-6 were isolated from Boston Harbor after enrichment on medium containing tributyltin (TBT) [16]. They were maintained on an estuarine salts agar [11] containing 10 ppm TBT.

Cells for experiments were first cultured in tryptone swarm agar [1]. After 24 h incubation at 27 °C, a loopful of cells from the edge of the swarm was transferred to 5 ml of tryptone broth and incubated overnight at 27 °C with shaking. Then 0.1 ml of this culture was transferred to 10 ml of tryptone broth in a Nephelo culture flask (Bellco Glass, Vineland, NJ) and incubated at 27 °C with shaking. When the turbidity reached 0.2 (about 10^8 cells ml^{-1}), cells from 5.0 ml of culture were harvested by centrifugation at 3400 × *g* at room temperature and washed three times in a solution which contained K_2HPO_4 1.063 g, KH_2PO_4 0.531 g and NaEDTA 0.037 g per liter of MilliQ water, final Ph 7.0, and suspended in that buffer without EDTA. At each step cells were suspended by rocking the tube gently by hand.

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Motile cells were determined before and after washing by a modification of a method described by Malmcrona-Friberg et al. [9]. Ten microliters of a 2.5% solution of glutaraldehyde was added to 2.0 ml of bacterial suspension. After 20 min the total number of cells was counted using a Levy counting chamber (Hausser Scientific, Cat. No. 500) (Hy-Lite Counting Chamber, Fisher Scientific, Pittsburgh, PA). The number of nonmotile cells was determined in a parallel sample without glutaraldehyde. The percentage of motile cells in the population was determined from the relation {[number of cells (+GA) − number of nonmotile cells (−GA)]/number of cells (+GA)} × 100.

Chemotaxis assays

In the modified method a disposable 1-μl capillary pipette (Drummond 'Microcaps', Fisher Scientific) was inserted through a hole made with an 18-gauge needle in a collar which consisted of the solid portion cut from a small serum stopper. As an alternative to the rubber stoppers, the end of the bulb supplied with the micropipettes can be used. The tube was sealed at one end by heating it in a bunsen flame and the open end was placed in a 10-ml beaker containing 0.01 M phospate buffer, Ph 7.0, or buffer containing attractant. As the tube cooled, the solution was sucked into the capillary.

The assay chamber (Fig. 1) consisted of a 96-well PVC assay plate and lid (Falcon Nos. 3911 and 3913 Microtest III). A hole was made in the lid over each well using a hot 18-gauge needle. Excess material which protruded on the inside of the lid was removed with a sharp knife. Wells in alternate rows were used to facilitate manipulation of the capillaries. Each well received 0.25 ml of the suspension of washed cells. Capillaries containing buffer or buffer and attractant were inserted through the holes, with the collar on the outside of the lid and the open end of the capillary extending into the solution in the well.

After 60 min the lid was lifted and the outside of all the capillaries was washed simultaneously by immersion in a small dish containing sterile water. The closed end was broken off each capillary and, with the aid of a small bulb supplied with the pipettes, the contents were expelled into a 1.5-ml Eppendorf tube containing 1.0 ml of tryptone broth. The contents of the tube were mixed and serial 10-fold dilutions were prepared in tryptone broth and triplicate spread plates were prepared from appropriate dilutions on the surface of tryptone agar. Plates were incubated at 27 °C for 24 h and colonies were counted.

Between assays the PVC plates were rinsed in ethanol, washed with soap and water, allowed to air dry and sterilized by exposure to a germicidal ultraviolet lamp.

When using Palleroni chambers, 0.4 ml of the bacterial suspension was pipetted into each of the four chambers of a sterile plate, taking care to avoid bubbles. A micropipette filled with attractant and with both ends open was placed in the channel of each chamber so that its tips were centrally located in each compartment. After 60 min the capillary was removed and the outside was washed with a thin jet of sterile water, proceeding from the center to the end. The capillary was then inverted and the other end was washed in the same manner, taking care that the stream of water did not strike the upper tip of the capillary and force out the contents. The contents were then expelled into tryptone broth, diluted and plated as above.

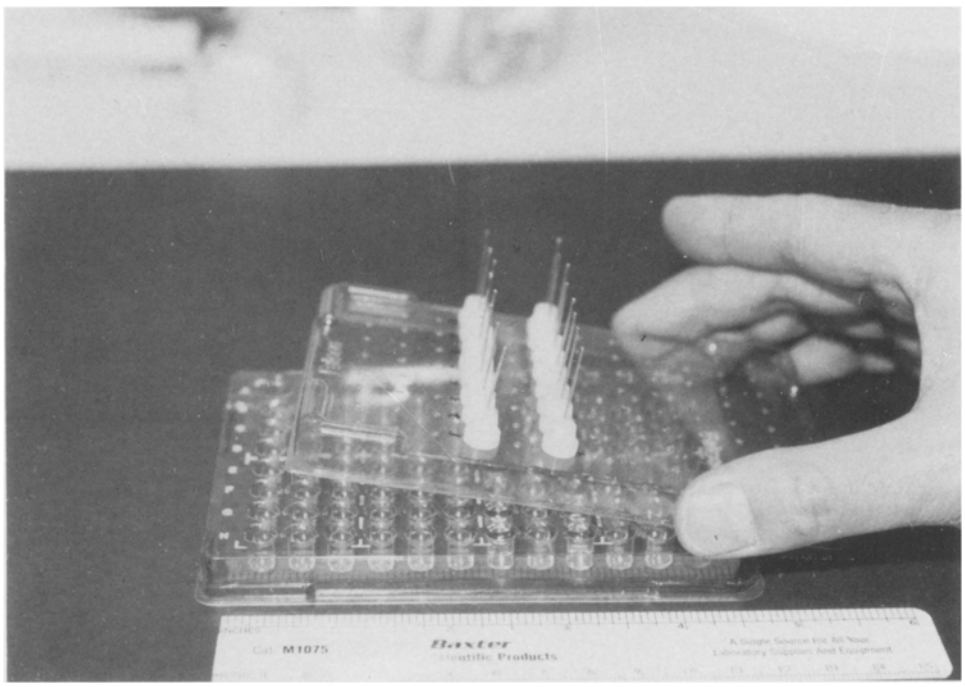

Fig. 1. Assay plate and cover with capillaries in place. For ease of manipulation capillaries are spaced in alternate rows.

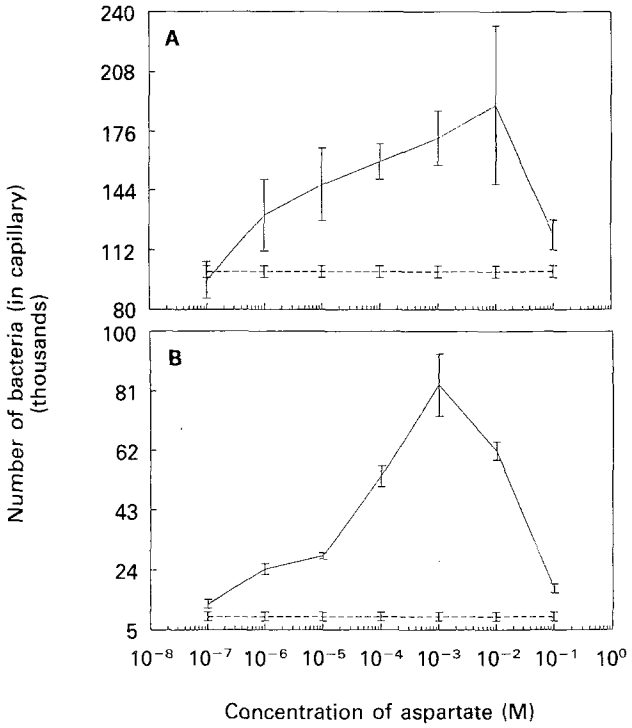

Fig. 2. Typical results for chemotaxis of *Pseudomonas* sp. SHC-6 toward aspartate: **A**, using Palleroni chamber; **B**, using assay plate. The dashed line represents accumulation of cells in capillary tubes containing buffer without attractant. Results are from triplicate assays.

RESULTS AND DISCUSSION

Cultures from shaken flasks generally contained 35–55% motile cells. After the washing procedures and suspension in chemotaxis medium there were about 10^8 cells ml^{-1} and about 10–30% of the population was motile.

Aspartate was a positive chemotactic agent for each of the organisms tested (Fig. 2). Variations in counts were consistently lower when the assay plate was used than with the Palleroni chamber. Actual numbers of cells in the capillary are greater when the Palleroni chamber is used because cells swim into both ends of the capillary rather than just one end in the modified method. Although typical results are presented, we have repeated the experiment three or more times by each method for each organism with the same results. Precision is particularly important in assays for chemotaxis because the number of bacteria which swim into the capillary is often only about two to four times the number which swim into control tubes. Therefore, variance must be limited in order to demonstrate statistically significant differences.

It is markedly easier to manipulate the capillary tubes with forceps when they are in the rubber collar than when they are without the collar because the collar is easier to grasp than the capillary itself. In addition, washing the outside of the capillaries is faster and less tedious when all are washed at the same time without having to pick up each tube individually. As with the Palleroni chamber, setting up the experiment is faster than in the original U-tube method since the chambers to contain the bacterial suspension are preformed, removing the necessity to assemble an individual chamber for each capillary. In addition, since all capillaries are immersed in the pond at the same time and removed at the same time, errors caused by variations in time of immersion associated with handling the capillaries with forceps are reduced greatly.

REFERENCES

1 Adler, J. 1966. Chemotaxis in bacteria. Science 153: 708–716.
2 Adler, J. 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis by *Escherichia coli*. J. Gen. Microbiol. 74: 77–91.
3 Adler, J. 1987. How motile bacteria are attracted and repelled by chemicals: an approach to neurobiology. Biol. Chem. Hoppe-Seyler 368: 163–173.
4 Adler, J. and M.M. Dahl. 1967. A method for measuring the motility of bacteria and for comparing random and non-random motility. J. Gen. Microbiol. 46: 161–173.
5 Allweiss, B., J. Dostal, K.E. Carey, T.F. Edwards and R. Freter. 1977. The role of chemotaxis in the ecology of bacterial pathogens of mucosal surfaces. Nature 266: 448–450.
6 Freter, R. and P.C.M. O'Brien. 1981. Role of chemotaxis in the association of motile bacteria with intestinal mucosa: in vivo studies. Infection and Immunity 34: 234–240.
7 Jones, C.J. and S.-I. Aizawa. 1991. The bacterial flagellum and flagellar motor: structure: assembly and function. Adv. Microb. Physiol. 32: 109–172.
8 Macnab, R.M. 1987. Motility and chemotaxis. In: *Escherichia coli* and *Salmonella Typhimurium*, Cellular and Molecular Biology, (Neidhardt, F.C., J.L. Ingraham, K. Brooks Low, B. Magasanik, M. Schaecter and H.E. Umbarger, eds) vol. 1, pp. 732–759, Amer. Soc. Microbiol., Washington, DC.
9 Malmcrona-Friberg, K., A. Goodman and S. Kjelleberg. 1990. Chemotactic responses of marine *Vibrio* sp. strain S14 (CCUG 15956 to low-molecular-weight substances under starvation and recovery conditions. Appl. Environ. Microbiol. 56: 3699–3704.
10 Manson, M.D. 1992. Bacterial motility and chemotaxis. Adv. Microb. Physiol. 33: 277–346.
11 Nelson, J.D. and R.R. Colwell. 1975. The ecology of mercury-resistant bacteria in Chesapeake Bay. Microb. Ecol. 1: 191–218.
12 Nevola, J.J., B.A.D. Stocker, D.C. Laux and P.S. Cohen. 1985. Colonization of the mouse intestine by an avirulent *Salmonella typhimurium* strain and its lipopolysaccharide-defective mutants. Infection and Immunity 50: 152–159.
13 Nikata, T., K. Sumida, J. Kato and H. Ohtake. 1992. Rapid method for analyzing bacterial behavioral responses to chemical stimuli. Appl. Environ. Microbiol. 58: 2250–2254.
14 Palleroni, N. J. 1976. Chamber for bacterial chemotaxis experiments. Appl. Environ. Microbiol. 32: 729–730.
15 Shonnard, D.R., R.T. Taylor, A. Tompson and R.B. Knapp. 1992. Hydrodynamic effects on microcapillary motility and chemotaxis assays of *Methylosinus trichosporium* OB3b. Appl. Environ. Microbiol. 58: 2737–2743.
16 Wuertz, S., C.E. Miller, R.M. Pfister and J.J. Cooney. 1991. Tributyltin-resistant bacteria from estuarine and freshwater sediments. Appl. Environ. Microbiol. 57: 2783–2798.