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Chamber for Bacterial Chemotaxis Experiments

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A design for a chemotaxis chamber and its use in bacterial chemotaxis experiments are described. Some of the advantages of the new design are discussed.

A convenient design for bacterial chemotaxis chambers is illustrated in Fig. 1. Four chambers have been excavated in a square Lucite plate measuring 5.5 cm per side and 1 cm in thickness. Each one of the chambers consists of two cylindrical compartments measuring 7 mm in diameter and 5 mm in height, linked by a channel 24 mm long, 2 mm wide, and 2 mm deep.

After appropriate cleaning, the plates are placed in individual petri dishes and exposed to ultraviolet radiation with the lid removed for sterilization. If desired, sterilization can also be done by autoclaving in glass petri dishes. A suspension of motile cells is pipetted to fill the compartments and the channel of each chamber. Bubbles in the chamber can be avoided by first filling one compartment, sliding the tip of the pipette along the edges of the channel while releasing the liquid, and finally filling the second compartment. Each chamber of the indicated dimensions is filled with about 0.4 ml of suspension.

As in the method proposed by Adler (1), a 1-μl disposable micropipette (Drummond Microcaps, Drummond Scientific Co.) is used for each

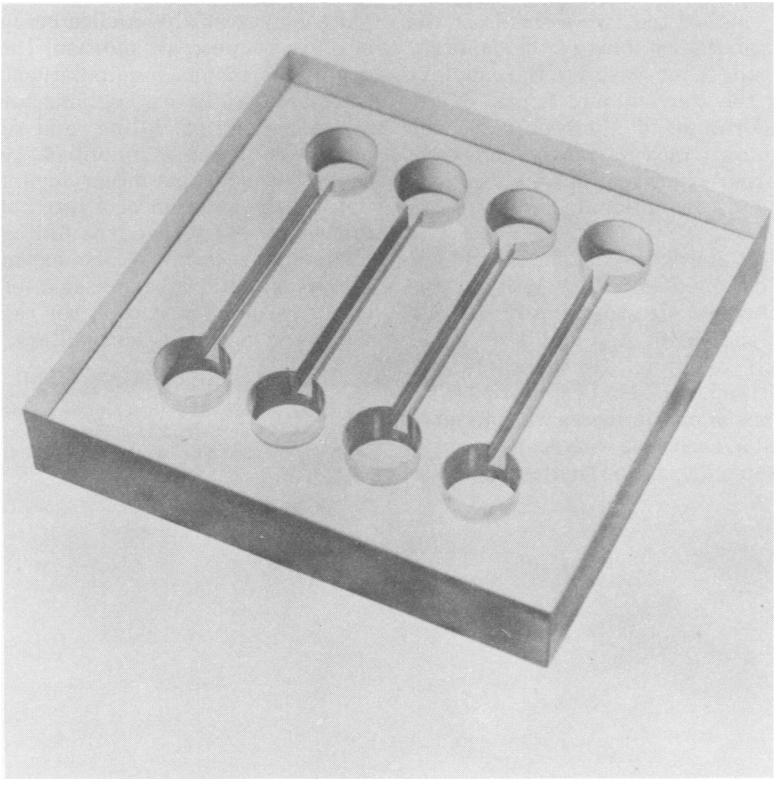

FIG. 1. *Four chemotaxis chambers in a Lucite plate.*

chamber, but instead of closing one end of each capillary, as in Adler's procedure, both ends of the capillary are left open in the present method. Clean capillaries that have been sterilized by dry heat are conveniently kept in petri dishes. Each capillary is picked up with thin tweezers, filled with the solution to be tested as attractant, and placed in the channel of a chamber containing the bacterial suspension, making sure that the capillary is immersed in the liquid and that its tips are centrally located in each of the terminal compartments. The chemotaxis plates in their individual petri plates are then placed in the incubator, and after the incubation period, each capillary is removed with the thin tweezers and washed externally with a thin jet of sterile water or buffer. The washing is performed in the following way. The capillary is held with the tweezers in a vertical position, and the jet of sterile liquid is directed to it at an angle, pointing downwards, so as to wash the capillary from the middle of its length to its lower tip. The jet is interrupted, the capillary is inverted, and the washing is repeated on the second half of the capillary, again washing from the middle to the lower tip. Care must be taken to prevent exposure of the upper tip of the capillary to the jet of liquid during each of the two sessions of the washing, so that the contents of the capillary are not forced out. After washing, the capillary is inserted in the conventional holder of the micropipette (Drummond Microcap), and the contents are blown into a known amount of sterile liquid from which appropriate dilutions are counted by conventional plating techniques.

Following the above precautions, excellent results were obtained experimentally in the study of the chemotactic properties of the motile spores of species of *Actinoplanes* (N. J. Palleroni, Arch. Microbiol., in press). In preliminary experiments, a set of capillaries filled with suspensions of motile spores was placed in chambers filled in each case with suspensions of spores of the same density as that contained in the capillaries. After incubation for a few minutes at 30°C, the capillaries were removed and washed externally as described above, and the spores were counted by plating. Working with two suspensions (6×10^9 and 3.6×10^6 spores/ml), the recoveries were within ±8% of the expected figures (coefficients of variation of 9 and 5%, respectively). The agreement breaks down at incubation times longer than about 10 min due to added complications of aerotactic reactions. Experiments performed in the above manner with nonmotile cells show good agreement for much longer periods of incubation. Control micropipettes filled with suspensions of known densities were subjected to washings without having been previously immersed in the bacterial suspension of a chemotaxis chamber, and the recoveries indicated a coefficient of variation of 4.5%. The results not only proved that the washing procedure was quite satisfactory, but also demonstrated that the insertion of the capillary into the conventional Microcap holder did not produce appreciable cell losses.

The chambers described here have been used in our laboratory for the experiments with *Actinoplanes* spores and they have been found to be extremely practical. In comparison with the chamber described by Adler (1), the chemotaxis plate is more easily handled because it is made of a single piece; in addition, the use of open capillaries makes manipulations less complicated because three operations, namely, closing of the capillaries, filling, and reopening, are either eliminated or simplified. The use of open capillaries adds one minor advantage: the volume of the solution of attractant is precisely known (in every case, the full capacity of the micropipette used for the experiment). This knowledge allows expression of the results either in number of cells per capillary or per unit volume within the capillary, if necessary.

LITERATURE CITED

1. **Adler, J.** 1973. A method for measuring chemotaxis and use of the method to determine optimum conditions for chemotaxis in *Escherichia coli*. J. Gen. Microbiol. 74:77–91.