



Note

A high-throughput capillary assay for bacterial chemotaxis

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Abstract

We present a high-throughput capillary assay in order to characterize the chemotactic response of the *E. coli* bacterium. We measure the number of organisms attracted into an array of 96 capillary tubes containing the attractant L-aspartate. The effect of bacterial concentration on the chemotactic response is reported. Such high-throughput assay can be used to characterize bacterial chemotaxis function of a wide range of biochemical parameters.

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Because bacteria are submerged in a patchy environment of naturally fluctuating biochemical parameters, it is reasonable to think that the chemotaxis system is not only able to integrate and respond to one stimulus at a time but rather to a wide range of multiple stimuli (Alexandre and Zhulin, 2001; Fenchel, 2002). Cells have to integrate and respond simultaneously to multiple input signals to survive (Bourret and Stock, 2002). In order to address this issue, we developed a high-throughput capillary assay to measure the chemotactic response function of wide range of biophysical and biochemical parameters. We demonstrate the validity of our approach by investigating the effect on the chemotactic response of a simple biophysical parameter, the cell density.

In the traditional capillary assay developed by Adler (Adler and Dahl, 1967; Mesibov and Adler, 1972; Adler, 1973), bacteria are suspended in a motility medium that does not support growth. A capillary tube containing a solution of an attractant is inserted into this suspension. A spatial gradient is formed by diffusion of attractant from the tip of the capillary tube. The bacteria migrate up this gradient and, some enter the tube. They are counted later by serial dilution and plating. The capillary assay is subject to variations, probably due to the convection of the attractant near the tip of the capillary tube (Berg and Turner, 1990; Ford and Lauffenburger, 1991). Moreover, at low cell concentration using serial dilutions and plating in order to count bacteria can be extremely tedious. Because of these technical constraints, the traditional capillary assay is a limited approach when chemotaxis needs to be characterized as a function of a large number of parameters.

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We modified the latter assay using 96-well microplates [800 μl UniFilter, Whatman] for which each well ends with a long drip director that acts as capillary. In order to fill up the 96 capillaries, we lower the capillaries into a filling array that has half number of its wells filled with motility medium, and the other half filled with motility medium and L-aspartic acid (attractant) at 10^{-2} M (Mesibov and Adler, 1972). This setup stands at 30°C for 40 min to allow the motility medium to be drawn into the capillaries. We subsequently added $150\ \mu\text{l}$ of molten wax to the tops of the capillaries in order to seal the attractant within. We allowed this setup to stand for a few minutes until the wax had fully cooled to 30°C (Fig. 1).

In order to perform the motility assay, we transferred the capillary array to a new microplate filled with varying concentrations of bacteria (Fig. 1). Each well was filled with $200\ \mu\text{l}$ of the bacterial suspension. The capillary array was allowed to incubate for 40 min at 30°C . After the incubation, the capillary array was rinsed so as to eliminate any remaining drops of bacterial media that might be adhering to the outside of the capillary tips.

The content of the capillaries were centrifuged down to a $350\ \mu\text{l}$ 96-well plate containing $200\ \mu\text{l}$ of LB broth. This assembly was centrifuged at 3000 rpm for 5 min in order to empty the capillaries into

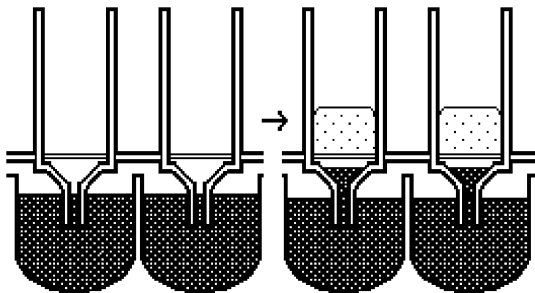


Fig. 1. In order to fill up the 96 capillaries, we lower the capillaries into a filling array that has half number of its wells filled with motility medium, and the other half filled with motility medium and L-aspartic acid (attractant) at 10^{-2} M. This setup stands at 30°C for 40 min to allow the motility medium to be drawn into the capillaries. We subsequently added $150\ \mu\text{l}$ of molten wax to the tops of the capillaries in order to seal the attractant within. Then, the 96 capillaries are transferred to a new microplate filled with various concentrations of bacteria. The distance between the mouth of the capillaries and the bottom of the wells is about 7 mm.

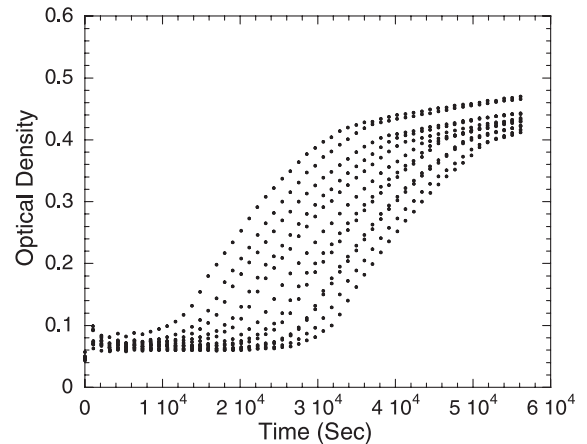


Fig. 2. Twelve typical growth curves of bacteria growing in individual wells of a 96-well plate. The time shift between growth curves depends on the initial cell concentration in each well. On this figure, the curve on the far left corresponds to an initial inoculation of $10\ \mu\text{l}$ at 10^7 cells/ml in $150\ \mu\text{l}$ of LB medium. As the curves shift to the right, the initial cellular concentration is diluted by successive power of two.

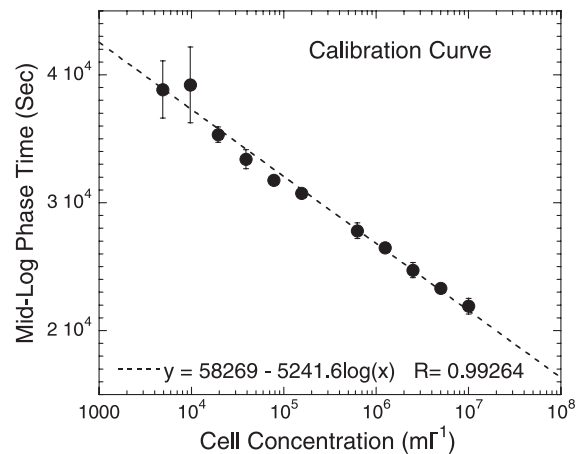


Fig. 3. Calibration curve. We calibrate the relationship between time shift and initial cell concentration by growing back solutions of cells with a known concentration. Cells are grown in individual well of a 96-well plate and time points corresponding to the mid-log phase are reported in the figure. The error bars represent the standard deviations from triplicate measurements. We find that the time shift decreases logarithmically with the initial cell concentration. In particular, we establish the following relationship: time shift = $a - b \times \log(\text{concentration})$. The best fit (dashed line) gives $a = 58269$ s and $b = 5241.6$ s.

the collection 96-well plate. Filtered light mineral oil (150 μ l) was then added to seal each well. The bacteria were grown back overnight at 37 °C into a Victor² 1420 multilabel plate reader (Perkin-Elmer) (Kalir et al., 2001). The plate reader measures the optical density of each well at set intervals and output a data file with the corresponding well numbers, optical densities, and timestamps. We set the reader to analyze the light absorbance at $\lambda = 600$ nm in each well, and then to shake the tray for 900 s in order to maintain homogenized cell growth in the wells. The plate reader repeats this cycle 54 times. Throughout the entire process, the plate is kept at a constant 37 °C.

A set of 96 consistent and reproducible growth curves is obtained from the overnight culture grown in the plate reader. We find that the time shift between each growth curve is a function of the original number

of cells in each different well. Fig. 2 shows growth curves obtained from 12 wells where the concentration of cells is diluted by successive power of two. We calibrate the relationship between time shift and number of cells by recording the time required for the optical density from each well to reach mid-log phase. This corresponds in our experiment as the region where the optical density is measured to be ~ 0.25 . Together with this criterion we establish a calibration curve by growing a known concentration of cells in each well (Fig. 3). We find that the calibration curve is well fitted with the following relation:

$$\text{Lag time} = a - b \times \log(\text{cell concentration}) \quad (1)$$

where a and b are the fitting parameters. Counting the bacteria using our calibration curve gave similar results than the plating method.

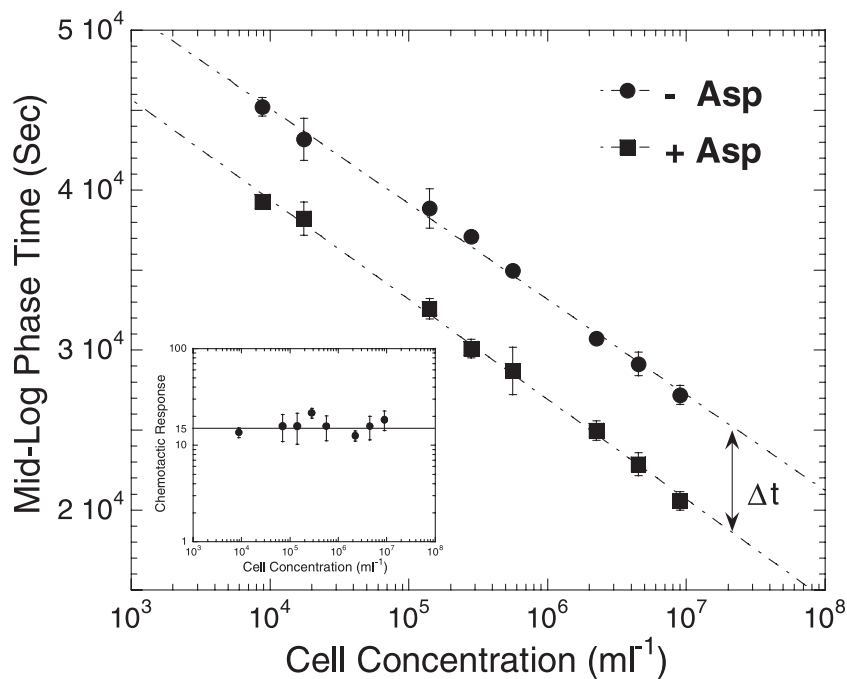


Fig. 4. Bacteria that had accumulated for 40 min at 30 °C in an array of 96 capillaries filled with motility medium (circles) and L-aspartate (10^{-2} M), when present (squares). Bacteria are grown back in a 96-well plate. We plotted time points corresponding to the mid-log phase in each well against the concentration of bacteria outside the capillary initially. A bacterial concentration of 6×10^8 /ml corresponds to an optical density of 1.0 at 600 nm. The number of bacteria accumulating by pure diffusion in the capillaries is directly proportional to the concentration of initially in solution (Adler, 1973). Inset: effect of the bacterial concentration on the chemotactic response. The chemotactic response is defined as the ratio of bacteria accumulated in the capillaries in the presence of attractant to accumulation in its absence: chemotactic response $R = 10^{\Delta t/b}$. This response is plotted against the concentration of bacteria outside the capillary initially. The plateau corresponds to a steady chemotactic response with amplitude of about 15.

We quantify the chemotactic response by measuring the time shift (Δt) between bacteria exposed to attractant versus the ones that are exposed to motility buffer only. When we inverse the relation (1) we can infer the following relationship between the concentration of cells in the capillary in presence of attractant (C_+) or not (C_-):

$$\text{Chemotactic response } (R) = (C_+/C_-) = 10^{\Delta t/b} \quad (2)$$

We find under these conditions that the amplitude of the chemotactic response, R , [number of bacteria accumulated in the capillaries in the presence of aspartate compared to its absence] is of the order of 15 (Fig. 4). This response is in agreement with previous studies with capillaries of identical inner diameter (0.5 mm) (Adler, 1973). Should the capillaries have a smaller inner diameter (0.2 mm) as noted by Adler the response would be larger (about 60). In earlier studies (Berg and Brown, 1972), it was found that a cloud of cells formed near the mouth of the capillary. At high cell density (about 10^8 cells/ml) this cloud sinks and affects the measured chemotactic response. In our work, measurements were performed at much lower concentrations (the maximum was 10^7 cells/ml) and were not perturbed by this effect.

In our experiment, we used the AW405 strain of *E. coli*, preserved in a frozen culture held at -70°C . Cells are extracted from this supply in a stab and are grown overnight in 3 ml of tryptone broth in a shaker at 35°C and 200 rpm. One hundred twenty microliters of this culture are then diluted into 6 ml of tryptone broth and further incubated at 35°C until an optical density between 0.35 and 0.50 is obtained. At that time, two samples of 1 ml are extracted and centrifuged at 3000 rpm for five min. The supernatant is discarded and the pellet is suspended in 1 ml of motility medium (Aswad and Koshland, 1974) (7.5×10^{-3} M $(\text{NH}_4)_2\text{SO}_4$, 6.0×10^{-2} M K_2HPO_4 , 2.0×10^{-3} M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, O, 1.3×10^{-4} M EDTA, 4.0×10^{-4} M L-methionine, 1.8×10^{-5} M FeSO_4) by gentle rocking. This procedure is repeated twice. At that time, the samples are diluted in 9 ml of motility medium and analyzed for optical density. From this measurement, the solution is further diluted to achieve the desired initial optical density of 0.1, corresponding to a cell concentration of 10^8 cells/ml (Adler, 1973).

Our experiment consists in running simultaneously 96 capillaries each of them being similar to Adler's (1973) assay. This assay is based on the motility of bacteria. It measures bacteria's ability to detect and to swim upward gradient of attractants. The number of bacteria accumulated in the capillaries in the presence of attractant is compared to accumulation in its absence. The ratio of these numbers is defined as the chemotactic response. A test-bed experiment is carried out to characterize the effect of the cell density on the chemotactic response. Previous investigations already characterize the effect of the cell density but are limited at high-to-medium density (Adler, 1973; Lewus and Ford, 2001). Low cell density is particularly tedious for conventional assays because statistical fluctuations of the number of cells have large amplitudes. With a high-throughput capillary assay we are able to characterize the chemotactic response across four orders of magnitude of the cell density (Fig. 4). As expected (Adler, 1973), we find a robust regime for a cell density ranging from 10^4 to 10^7 cells/ml (Fig. 4, inset). Over this range the chemotactic response was conserved.

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