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Hydrodynamic Effects on Microcapillary Motility and Chemotaxis Assays of *Methylosinus trichosporium* OB3b

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A study of the random motility and chemotaxis of *Methylosinus trichosporium* OB3b was conducted by using Palleroni-chamber microcapillary assay procedures. Under the growth conditions employed, this methanotroph was observed qualitatively with a microscope to be either slightly motile or essentially nonmotile. However, the cells did not not respond in the microcapillary assays in the manner expected for nonmotile Brownian particles. As a consequence, several hydrodynamic effects on these Palleroni microcapillary assays were uncovered. In the random-motility microcapillary assay, nondiffusive cell accumulations occurred that were strongly dependent upon cell concentration. An apparent minimal random-motility coefficient (μ) for this bacterial cell of 1.0×10^{-7} cm^2/s was estimated from microcapillary assays. A simple alternative spectrophotometric assay, based upon gravitational settling, was developed and shown to be an improvement over the Palleroni microcapillary motility assay for *M. trichosporium* OB3b in that it yielded a more-accurate threefold-lower random-motility coefficient. In addition, it provided a calculation of the gravitational-settling velocity. In the chemotaxis microcapillary assay, the apparent chemotactic responses were strongest for the highest test-chemical concentrations in the microcapillaries, were correlated with microcapillary fluid density, and were strongly dependent upon the microcapillary volume. A simple method to establish the maximal concentration of a chemical that can be tested and to quantify any contributions of abiotic convection is described. Investigators should be aware of the potential problems due to density-driven convection when using these commonly employed microcapillary assays for studying cells which have low motilities.

Microcapillary assay methods have been used extensively to measure the random motility and chemotaxis of bacteria (1–4, 13–17, 19). In these assays, the end(s) of a microcapillary containing motility or chemotaxis medium is exposed to cells suspended in the motility medium and the number of bacteria entering the microcapillary is determined as a function of time by using either viable counting, electronic particle counting, or direct microscopic counting methods. The results from random-motility assays have been used to determine the cell population motility coefficient by comparison with mathematical models. In chemotaxis experiments, a gradient of the chemoattractant develops between the internal fluid within the ends of the microcapillary and the external cell suspension. Increased accumulations of cells within the microcapillary containing the chemoattractant confirms a positive chemotactic response. Results from these types of microcapillary experiments have been useful for establishing medium compositions for motility measurements, screening for chemoaffectors, and determining threshold concentrations for chemotactic responses.

Recently, however, microcapillary assay methods have been criticized because the experimental results must be interpreted in the absence of well-defined bacterial and chemical gradients (7, 11, 12, 26), although the effects of these nonidealities on the assay results have not been quantified. For example, in random-motility assays, the ends of a microcapillary filled with the motility medium are immersed in a suspension of cells in this same fluid. Because of some entrainment of the cell suspension fluid during immersion, there is likely an ill-defined initial gradient in the cell concentration at the microcapillary ends. Similarly, in chemotaxis experiments, there are likely nonspecified initial gradients in both the cell concentration and the chemotaxis test agent. In addition, because of solution density differences between the microcapillary fluid and the cell suspension, there is a potential for cell accumulation inside the microcapillary due to hydrodynamic effects (density-difference-induced fluid flow) in both random-motility and chemotaxis experiments.

We have measured quantitatively several of the previously mentioned hydrodynamic effects that can occur during Palleroni-chamber (19) microcapillary assays of bacterial random motility and chemotaxis. Such assays were applied to the obligate methanotroph *Methylosinus trichosporium* OB3b, which is known to biodegrade a number of chlorinated aliphatic hydrocarbons (18, 20, 24, 25). Interest in this methanotroph stems from its potential use in the bioremediation of contaminated groundwater and, consequently, a desire to obtain information about its random-motility and possible chemotactic responses to select target compounds, including groundwater pollutants. In this article, we describe the behavior of this methanotroph during a set of random-motility and chemotaxis experiments. We attribute the results mainly to abiotic hydrodynamic effects, which have generally gone unrecognized during the utilization of Palleroni-chamber microcapillary assays.

MATERIALS AND METHODS

Cell culture. Cells were grown to a density of approximately 5×10^8 ml^{-1} at 30°C and at a shaker speed of 300 rpm in 20-ml volumes of medium contained in sealed 250-ml side-arm flasks (20). Methane and air in equal volumes were available to the suspension culture from the headspace of the flask. The growth medium was Higgin's nitrate minimal salt

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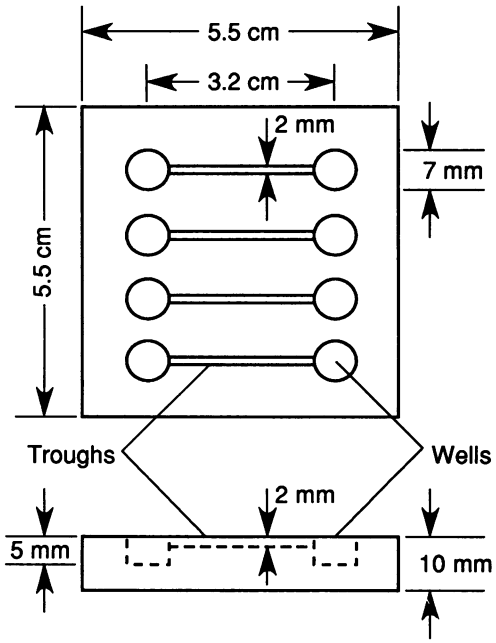

FIG. 1. Schematic diagram of the Plexiglas Palleroni chamber. Microcapillaries (32 mm long; 5, 3, or 1 μl) were placed horizontally with reference to gravity in the troughs (25 by 2 by 2 mm). The ends of the microcapillaries extended into the chamber wells which contained the cell suspensions.

medium (pH 7.0) (9) from which $CuSO_4$ was deleted, HNMS(−Cu) (20). Cells were harvested in the late-log or early stationary growth phase after 24 h of incubation, centrifuged at 12,000 × *g* for 5 min at 20°C, resuspended twice in filtered HNMS(−Cu) (0.22-μm-pore-size cellulose acetate membrane; Corning, Inc.), and then used immediately for the various experiments. In some cases, where indicated, aliquots of the cells in HNMS(−Cu) were heat killed by warming them to 50°C for 20 min.

Periodically, the freshly prepared bacterial suspensions were examined at a magnification of ×1,200 with a phase-contrast microscope. Qualitatively, very little or no cell motility was seen in comparison with the motility of 2-μm-diameter polystyrene microbeads (Duke Scientific).

Random-motility microcapillary assay. The Palleroni-chamber (19) and microcapillary assay methods were used to determine initial values for the cell random-motility coefficient and to measure all of the chemotactic responses. The latter are described below (see "Chemotaxis microcapillary assay"). The chamber dimensions are diagrammed in Fig. 1. For the motility coefficient measurements, 0.45 ml of a cell suspension in filtered HNMS(−Cu) and at a known concentration was added to the wells of the Palleroni chamber. Routinely, 5-μl glass microcapillary tubes (length, 32 mm; inside diameter, 44.5 μm; Drummond Scientific Co.) containing filtered HNMS(−Cu) were placed in the troughs of the chamber at the start of the experiment. After 1, 2, 3, and 4 h, three microcapillaries were removed, and their contents were expelled into a single 3.0-ml volume of triple-filtered saline counting solution (4% NaCl). The cells were enumerated by using a Coulter electronic particle counter (model ZBI) having a 30-μm aperture, and the cell size distribution was recorded by using a Coulter Channelyzer and an x-y recorder. Temperature and pH were routinely 23°C and 7.0, respectively.

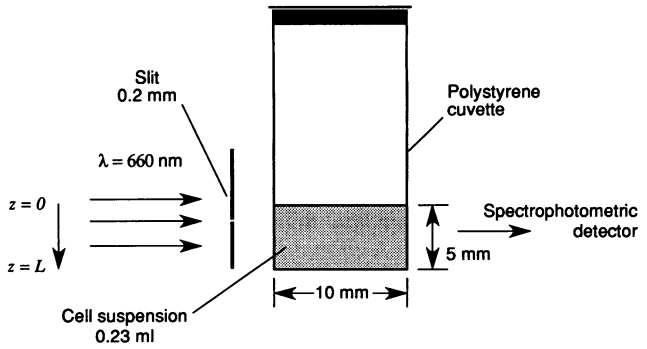

FIG. 2. Schematic diagram of the gravitational-settling assay cuvette.

Minor but important manipulative changes in the standard Palleroni microcapillary assay (19) consisted of wiping the external surfaces of the microcapillaries with both moist and dry tissues instead of using a stream of distilled water to remove externally adhering cells. In addition, the contents of the microcapillaries were expelled vigoriously with multiple rinsing into the counting solution by using a mechanical pipette dispenser (Drummond). Control experiments consistently showed that unless the pipette-dispenser was used, only about 70% of the cells in the microcapillaries could be recovered after 2 to 3 h of incubation because of attachment of the bacteria to the internal glass microcapillary walls over time.

Spectrophotometric random-motility and gravitational-settling assay. An independent alternative method for obtaining the random-motility coefficient of low-motility cells was developed and also applied to *M. trichosporium* OB3b. It is based upon the gravitational settling and random diffusion of cell suspensions within an isothermal cuvette. Cell settling was monitored by recording the change in absorbance of visible light as the settling front passed a narrow slit located 1.5 mm below the suspension surface (Fig. 2). A 0.23-ml volume of cell suspension at 5×10^8 ml^{-1} in HNMS(−Cu) (pH 7.0) was added to a modified polystyrene cuvette (Bio-Rad Laboratories). The cuvette was machined to fit an isothermal cuvette holder (23°C; Gilford Instrument Corp.), and it was cut to a height of 2.0 cm. The modified cuvette (0.5 ml) was capped with a rubber seal and then placed in an isothermal cuvette holder within a Gilford 260 UV-visible spectrophotometer. The use of a cap prevented evaporation, and the use of polystyrene minimized meniscus formation. A 0.2-mm light slit for detecting changes in suspension absorbance was created by applying plastic tape to the exterior of the cuvette holder. The time course of the decrease in A_{660} was recorded continuously with a Gilford 6051 recorder. Simultaneous calculations of the gravitational-settling velocity and the random-motility coefficient were performed by a visual fit of the data to a numerical solution of the convective-diffusion equation of cell population transport in the cuvette,

$$\frac{\partial b}{\partial t} + v_y \frac{\partial b}{\partial z} - \mu \frac{\partial^2 b}{\partial z^2} = 0 \quad (1)$$

$$t = 0 \qquad 0 \le z \le L \qquad b = b_0 \quad (2)$$

$$t > 0 \qquad z = 0 \qquad v_y b = \mu \frac{\partial b}{\partial z} \quad (3)$$

$$t > 0 \quad z = L \qquad v_y b = \mu \frac{\partial b}{\partial z} \qquad (4)$$

where b is the cell concentration (in cells per cubic centimeter), t is time (in seconds) v_y is the settling velocity of the cell suspension (in centimeters per second), z is the depth of the suspension within the cuvette (in centimeters), and μ is the random-motility coefficient (in square centimeters per second). A Crank-Nicolson (8) formulation was used for the numerical solution.

Chemotaxis microcapillary assay. We surmised that fluid density differences between the cell suspension in the Palleroni chamber wells and the internal microcapillary solution would cause a flow of cells into the microcapillaries and could, thereby, confound chemotaxis measurements at high test-chemical concentrations. Vicker has shown that higher-density capillary fluids will flow from the ends of an immersed horizontal microcapillary and descend in a waterfall-like manner through another less-dense fluid (26). As a result, a set of experiments was carried out to ascertain the effects of an increasing concentration of the internal microcapillary solution on this microcapillary flow and on the accompanying reverse flow of cells into the microcapillaries from the Palleroni chamber wells. Some of these experiments employed heat-killed cells to eliminate any biological response. Others used phenol red (a commonly used pH indicator in cell cultures; Sigma Chemical Co.), since it gave a response to the internal microcapillary solution concentration and density equivalent to that of heat-killed cells. The pooled contents of 10 microcapillaries containing only HNMS(−Cu) were used as a control. The number of heat-killed cells or the amount of phenol red (200 μM) which entered these microcapillaries from the chamber wells was measured after 1 h. In the case of phenol red, the contents of 10 microcapillaries were expelled into a single 0.45-ml volume of water in a quartz microcuvette, and the A_{558} was measured after bringing the pH to 12 by adding 6 μl of 1.0 N NaOH.

The chemotactic responses of viable *M. trichosporium* OB3b to several sugars and amino acids (Sigma cell culture reagents), two chlorinated hydrocarbons (Aldrich; >99% purity), and methane (Airco Gas; 99.99% purity) were measured by using the same procedure outlined above for the motility coefficient experiments, except that in addition to filtered HNMS(−Cu) in the microcapillaries, the chemotaxis test agent was also present. The test-chemical concentrations ranged from 10^{-8} to 10^{-2} M. The number of cells which entered the microcapillaries after 1 h was measured and compared with the number which entered in the absence of the test chemical. Three microcapillaries were used for each test-chemical concentration, and after 1 h, their contents were expelled into a single 3.0-ml volume of 4% NaCl for cell enumeration by using a Coulter counter. Each chemotaxis experiment with viable bacteria was replicated at least once. These experiments were conducted with a cell concentration of 5×10^8 ml^{-1} at 23°C and pH 7. For the volatile chlorinated compounds (trichloroethylene [TCE] and chloroform), stock solutions in HNMS(−Cu) were prepared so as to leave a negligible headspace in screw-capped 22-ml vials having polytetrafluoroethylene-faced red rubber septa (Kimball Glass Inc.). Storage and recovery analyses of the stock volatile solutions by gas chromatography established that their concentrations remained unchanged for up to 1 day. A 10-μl gas-tight syringe was used to introduce the TCE or chloroform solutions into the 5-μl microcapillaries that were employed for these chemotaxis experiments.

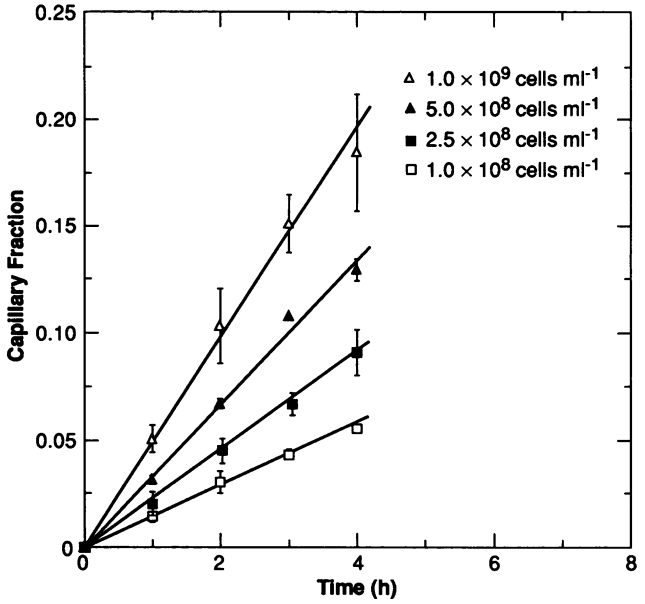

FIG. 3. Palleroni microcapillary data for the random motility of *M. trichosporium* OB3b at several cell concentrations in the chamber wells. Each datum point was obtained from an analysis of three pooled microcapillaries (5 μl each). Error bars were calculated by using the standard error of two independent experiments.

For the aerobic and anaerobic chemotaxis assays involving methane, aqueous solutions of HNMS(−Cu) were sparged vigorously in small volumes with methane and air (1:1 [vol/vol]) or with methane alone, respectively. After sparging, the concentrations of methane were determined by gas chromatography. An equal number of experiments were conducted in which 1 mM sodium formate was included in the test solution. As for the assays with TCE and chloroform described above, a 10-μl gas-tight syringe was used to introduce these methane-containing solutions into 5-μl microcapillaries.

RESULTS

Random motility of *M. trichosporium* OB3b. Horizontal microcapillary assays were performed, and the results were converted to a dimensionless number termed the capillary fraction. This is the ratio of the number of cells that accumulate inside the microcapillary after a given time divided by the number of cells in the microcapillary when it is filled with the chamber well cell suspension. Figure 3 shows the capillary fraction versus time for several cell concentrations in the chamber wells. There was a rather linear increase as a function of time at each cell concentration. Since diffusion models predict that the capillary fraction should increase with the square root of time (10), it is difficult to attribute these results exclusively to diffusion. Also, the capillary fraction increased with cell concentration at every time point. There is no basis in either motility or diffusion theory for this observation. Moreover, a control experiment utilizing heat-killed cells at 5×10^8 ml^{-1} resulted in nearly the same assay response (data not shown) as that for the viable cells. This confirmed that an abiotic mechanism must be responsible for most of the cell movement observed in Fig. 3, and it revealed that a horizontal microcapillary assay cannot distinguish between biotic and abiotic

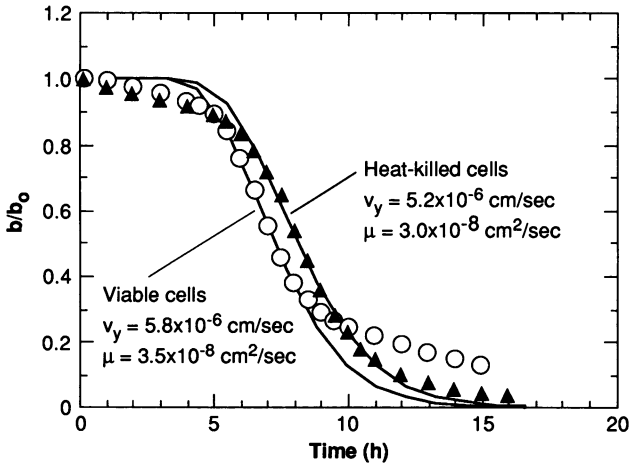

FIG. 4. Gravitational-settling data for viable and heat-killed *M. trichosporium* OB3b at a cell concentration of 5×10^8 ml^{-1} in HNMS(−Cu). b/b_0 are the normalized cell concentration values. From a model fit of the data (equations 1 to 4), values for the settling velocity (v_y) and the random-motility coefficient (μ) were calculated and are given in the figure.

responses for a cell with low motility. Since the magnitude of the cell accumulation, nevertheless, was still greater than that expected for the diffusion of an inert particle (21), another abiotic mechanism besides diffusion must dominate the observed accumulation shown in Fig. 3. The only other mechanism of abiotic cell movement which could account for these experimental results is convection (fluid flow of cells into the microcapillary), which is addressed in Discussion.

To circumvent the effects of convection on the determination of the random-motility coefficient, a spectrophotometric assay was developed. It was applied to *M. trichosporium* OB3b, although it should be useful for a wide variety of cells that have marginal or low motilities. Figure 4 displays a match of the numerical solution of equations 1 to 4 with the settling data for concentrations of 5×10^8 ml^{-1} of viable and heat-killed cells. The gravitational-settling velocity of the heat-killed cells was slightly smaller than that of the viable cells, suggesting that a slight size reduction of the cells occurred upon heating. Interestingly, the random-motility coefficients were nearly the same in both cases ($\sim 3 \times 10^{-8}$ cm^2/s), again indicating that the cells are, at most, only slightly motile. The slight deviations of the model from the data in Fig. 4 at both early and late times can be attributed to the polydisperse size of the cells, whereas in the model the cell size is assumed to be monodisperse.

Chemotactic response of *M. trichosporium* OB3b. Data from microcapillary chemotaxis experiments are often reported in the literature in terms of a ratio called the chemotaxis index (CI) (13, 16). It is the number of cells that accumulate after a given time, generally 1 h, within a microcapillary containing a chemotaxis test compound divided by the number of cells accumulated within a microcapillary lacking the compound. Arithmetically, it is also equivalent to the capillary fraction in the presence of the test agent divided by the capillary fraction in its absence. Values of CI significantly greater than 1 identify a positive response of the cells to the chemoaffector (chemoattraction), and values significantly less than 1 (but greater than 0) indicate a negative response (chemorepulsion).

High concentrations of commonly studied compounds such as sugars and amino acids can cause the microcapillary fluid to have a significantly higher density than the cell suspension in the Palleroni chamber wells, giving rise to density-driven flow out of and back into the microcapillary. Concerns about density-driven flows in random-motility and chemotaxis experiments have also been raised by other investigators (12, 26), but they have not been addressed quantitatively in terms of microcapillary cell accumulations. The effects of the microcapillary test-chemical concentration and fluid density on the number of cells entering the microcapillaries after 1 h were quantified by using phenol red or heat-killed cells, thus eliminating any biological response from the experiments. Table 1 shows that for two sugars and two amino acids at 10^{-1} and 1 M the microcapillary fluid density increased sharply, causing phenol red to accumulate from the Palleroni chamber wells into the microcapillaries to a much greater extent than would be expected for simple diffusion. Comparable data (not shown) were obtained for the same compounds shown in Table 1 when heat-killed cells were used. This density-induced flow of phenol red from the chamber wells decreased as the microcapillary diameter decreased, as shown by experiments using D-glucose as the test chemical (Fig. 5). As a result of these findings, all of our chemotaxis experiments using viable cells were devised so that the test chemoaffector concentrations did not exceed 10^{-2} M, thus eliminating microcapillary convection.

Experimental microcapillary CIs for *M. trichosporium* OB3b toward the same sugars and amino acids shown in

TABLE 1. Effects of microcapillary solution density on the reverse flow of phenol red from the Palleroni chamber wells into microcapillaries[a]

Test-chemical concn (M)	D-Glucose		D-Ribose		L-Serine		Glycine	
	MSD[b]	CF[c]	MSD	CF	MSD	CF	MSD	CF
0	0.9990	0.123 ± 0.010	0.9988	0.111 ± 0.012	0.9991	0.101 ± 0.010	0.9986	0.091 ± 0.007
10^{-3}	0.9995	0.119 ± 0.011	0.9994	0.127 ± 0.010	0.9993	0.092 ± 0.015	1.000	0.094 ± 0.013
10^{-2}	1.000	0.120 ± 0.011	0.9995	0.170 ± 0.009	0.9997	0.111 ± 0.016	0.9989	0.100 ± 0.014
10^{-1}	1.007	0.361 ± 0.008	1.006	0.288 ± 0.008	1.004	0.229 ± 0.016	1.003	0.142 ± 0.013
1	1.080	0.834 ± 0.002	1.057	0.939 ± 0.006	1.045	0.855 ± 0.006	1.026	0.429 ± 0.011

[a] Accumulation of the colored pH indicator, phenol red (well concentration, 200 μM), in 5-μl microcapillaries was measured spectrophotometrically in 0.5-ml quartz cuvettes (see Materials and Methods). The test chemicals for which data are shown are D-glucose, D-ribose, L-serine, and glycine.

[b] MSD, microcapillary solution density (in grams of test chemical solution per milliliter).

[c] CF, capillary fraction for phenol red, i.e., the ratio of A_{558} of 10 pooled 5-μl microcapillaries to that of 10 pooled microcapillaries containing 200 μM phenol red (the data are averages of replicate measurements ± standard deviations). Note that large apparent CIs for each test chemical, with respect to phenol red, would be obtained by dividing each CF entry for the 10^{-1} and 1 M concentrations by the corresponding 0 M capillary fraction.

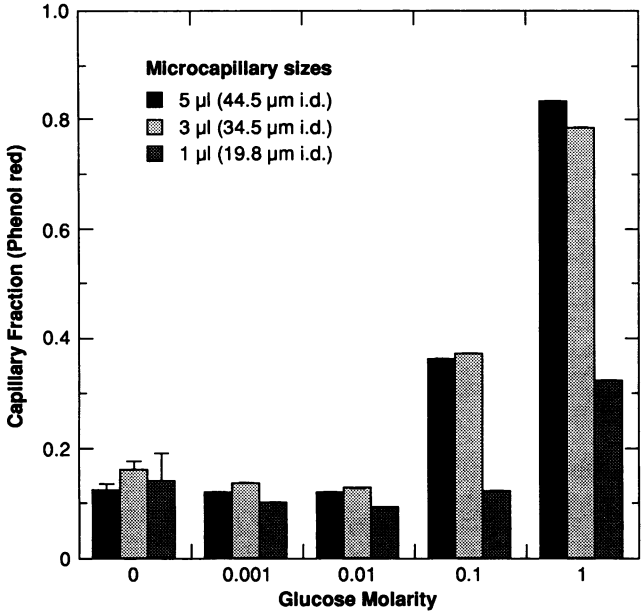

FIG. 5. Effects of microcapillary volume on the 1-h accumulation of phenol red [200 µM in HNMS(−Cu)] from the chamber wells during a Palleroni chemotaxis assay. Ten microcapillaries containing glucose dissolved in HNMS(−Cu) were utilized to obtain each data bar. Error bars are the standard deviation of replicate experiments at 0 M glucose.

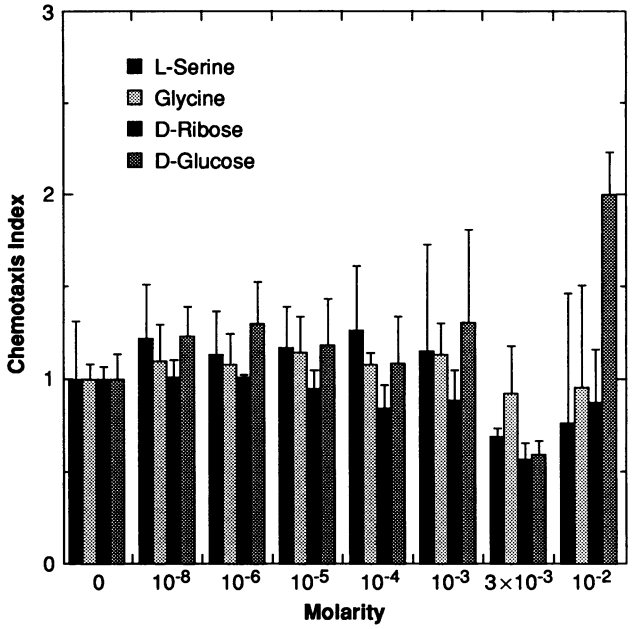

FIG. 6. Chemotactic responses of *M. trichosporium* OB3b to several sugars and amino acids in HNMS(−Cu) after 1 h by using the Palleroni microcapillary assay. The cell concentration was 5×10^8 ml^{-1}. Each data bar was obtained from an analysis of three pooled microcapillaries (5 µl each), and error bars were calculated by using the standard deviation of two experiments.

Table 1 are presented in Fig. 6 for the concentration range of 10^{-8} to 10^{-2} M. No measurable chemotactic effect was observed except for a slight chemorepulsion, which was detected for L-serine, D-ribose, and D-glucose at 3×10^{-3} M, and a small chemoattraction, which was measured for D-glucose at 10^{-2} M.

Microcapillary CIs with respect to TCE and chloroform are shown in Fig. 7. The CIs of the cells remained near 1 for the concentration range of 10^{-8} to 10^{-3} M, indicating no response. At a concentration of 5×10^{-3} M TCE and 10^{-2} M chloroform, however, there was a small but measurable reduction in the CIs (chemorepulsion).

The response of the cells to methane was also measured by using HNMS(−Cu) that was saturated with methane in the presence and absence of air (Table 2). The addition of formate, which stimulates whole-cell-soluble methane monooxygenase oxidative activity with aliphatic chlorinated hydrocarbons (5, 6, 18, 20, 24), was an additional parameter. No substantial chemotactic response to methane, air, or formate was detectable.

DISCUSSION

We have demonstrated that random-motility and chemotaxis experiments using Palleroni microcapillary assays are subject to abiotic hydrodynamic effects arising from a density-induced convection. This convection was shown to be dependent upon assay conditions such as the cell concentration, microcapillary test-chemical concentration, and microcapillary volume. The extent to which abiotic convection in microcapillary assays contributes to the measured random motility and chemotaxis is, in turn, dependent upon the inherent motility of the particular microbe being studied. Although these hydrodynamic effects in the microcapillary assays are not likely to obscure the motility and chemotaxis measurements of previously studied highly motile strains of bacteria (3, 14, 15, 17), they may account for a substantial fraction of the lower-magnitude chemotactic responses reported in the literature where microcapillary assays have been used (13, 16).

All of the random-motility data in Fig. 3 exhibited nondiffusive behavior in that there was a linear increase in capillary fraction with time for each cell suspension concentration and an increase in capillary fraction with increasing cell concentration at every time point. A true diffusion response should result in an increase in the capillary fraction with the square root of time, and there should be no dependence of capillary fraction on cell concentration for these dilute suspensions (10, 22). The abiotic convection which was most likely responsible for the cell movement observed in Fig. 3 could have been induced by the fluid density differences between the higher-density cell suspensions and the lower-density microcapillary motility buffer. In support of this conclusion, a buoyancy-driven flow of a cell suspension into a motility buffer with a lower fluid density has been observed by others (12).

Even though nondiffusive cell movements dominated the accumulation results in Fig. 3, an upper limit to the random-motility coefficient for *M. trichosporium* OB3b can be estimated by matching the one-dimensional diffusion model prediction (10) to the data in Fig. 3 for 10^8 cells per ml at the 1-h time point. This calculation gives a random-motility coefficient of 1.0×10^{-7} cm^2/s, while the Stokes-Einstein diffusion coefficient for an equivalently sized inert particle, taken for this estimate as a 1-µm-diameter sphere, is only 4×10^{-9} cm^2/s (21). Therefore, according to this microcapillary assay, the true random-motility coefficient of *M. trichosporium* OB3b in an aqueous solution of HNMS(−Cu) is constrained to lie between the limits of ($\sim 1 \times 10^{-7}$ cm^2/s) >

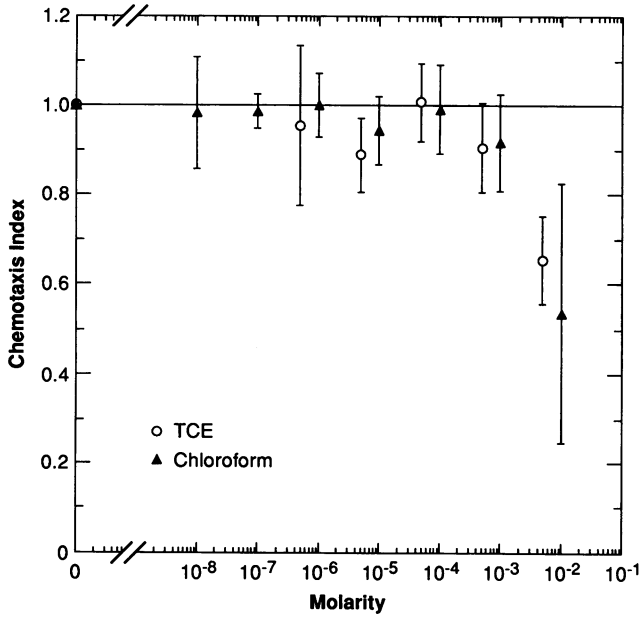

FIG. 7. Chemotactic responses of *M. trichosporium* OB3b to TCE and chloroform in HNMS(−Cu). The experimental conditions were the same as those described in the legend to Fig. 6. Each datum point was obtained from an analysis of three pooled microcapillaries (5 μl each), and error bars were calculated by using the standard deviation of two experiments. The horizontal line at a CI of 1 represents no chemotactic response.

$\mu \geq (\sim 4 \times 10^{-9}$ cm^2/s). This range of values for μ is at least 2 orders of magnitude below those reported previously for highly motile strains of bacteria (4, 12, 22).

The value of μ determined for *M. trichosporium* OB3b from the spectrophotometric gravitational-settling assay was $\sim 3 \times 10^{-8}$ cm^2/s (Fig. 4), while the lower limit for random-motility coefficient determinations with the microcapillary assay appears to be near 10^{-7} cm^2/s. Thus, this simple alternative motility assay offers a threefold-lower limit than that of the microcapillary assay for μ determinations of relatively nonmotile cells. Moreover, from the same experimental data, a value for the gravitational-settling velocity also can be calculated.

The chemotaxis experiments revealed that above a test-compound concentration of 10^{-2} M, the density of the microcapillary fluid increased sharply, as summarized in Table 1 for several sugars and amino acids. This was accompanied by corresponding increases in the reverse flow of fluid from the Palleroni chamber wells. These experiments established that there is an upper concentration limit for a given test chemical within the microcapillary, which can be studied without creating a large false-positive abiotic increase in the CI. Moreover, an upper limit for its concentration exists, irrespective of whether the chemical being tested is a potential chemoattractant or a chemorepellent. A simple procedure to quantify this convective interference and to define the maximal permissible test-chemical level was outlined. It involves the use of phenol red, which can be measured spectrophotometrically, or heat-killed cells, which can be counted electronically.

Palleroni-chamber microcapillary assays are much more suitable and accurate for highly motile microorganisms since the hydrodynamic convective effects become a much smaller fraction of the total response. Use of this assay with low-motility cells may be a problem because convective effects can dominate the assay response. In cases where preliminary microcapillary data yield a low apparent motility coefficient, i.e., $\sim 10^{-7}$ cm^2/s, the random motility should be carefully examined further as a function of cell concentration and by a direct comparison of viable and nonviable (i.e., heat-killed) cells. If the motility assay response (capillary fraction) increases with cell concentration and viable and nonviable cells behave almost identically, then a likely basis for most of the observed microcapillary accumulation data is convection. A potential solution to the convection problem in motility studies is to use the lowest cell concentration and the smallest microcapillary size that the cell enumeration method will allow, since it was shown that convection is reduced with decreasing cell suspension concentration and microcapillary sizes (Fig. 3 and 5). Yet, even in the presence of convection, the range of values in which the true random-motility coefficient must lie can be determined, and an independent estimation of μ can be made by a simple spectrophotometric gravitational-settling assay.

TABLE 2. Effect of methane on the chemotactic response of *M. trichosporium* OB3b in HNMS(−Cu)

Dissolved $[CH_4]$ (M)[a]	Culture conditions		CI[b]
	Sodium formate[c]	Air[d]	
0	−	+	1.00 ± 0.11
1.27×10^{-3}	−	−	1.04 ± 0.07
7.10×10^{-4}	−	+	0.95 ± 0.04
0	+	+	1.00 ± 0.11
1.44×10^{-3}	+	−	0.90 ± 0.12
6.60×10^{-4}	+	+	0.90 ± 0.06

[a] Measured by gas chromatography.
[b] Averages of two experiments ± standard deviations.
[c] Sodium formate (1 mM) was (+) or was not (−) added.
[d] Response was measured in HNMS(−Cu) saturated with methane in the presence (+) or absence (−) of air.

By using the Palleroni-chamber microcapillary assay, *M. trichosporium* OB3b was determined to be only slightly motile or essentially nonmotile and it appears to lack the ability to migrate appreciably toward chemical stimuli. A lack of motility and chemotaxis in microorganisms which have favorable metabolic properties for the in situ bioremediation of polluted aquifers may be advantageous in some cases. In an application such as an in situ microbial filter technology, cell attachment to subsurface material would be crucial. In this situation, a very active and prolonged cell motility could be incompatible with the intended immobilized state of the cells (23).

Rapid, simple, and inexpensive methods for quantitatively screening newly isolated environmental microorganisms for either their random motility or their chemotactic properties can be important. Palleroni microcapillary assays, when used properly, can be one such method. However, their lower limits of reliability must be recognized and carefully checked, especially when dealing with weakly motile or essentially nonmotile bacteria.

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