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ORIGINAL RESEARCH ARTICLE

# A Novel Device and Method for Assay of Bacterial Chemotaxis Towards Chemoattractants

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**Abstract** Capillary assemblies and microfluidic devices used for bacterial chemotaxis assays have certain inherent limitations. This opens opportunities for innovation in the area. The present study describes an innovative economical device called chemotaxis plate and also a method to use this device for chemotaxis assay. Two type cultures, *Pseudomonas putida* MCC 2989 and *Bacillus subtilis* MCC 2049, chemotactic to L-aspartate, were used to validate the new device and establish the protocol for assay. 100 to 1000 fold higher number of cells were recovered in presence of chemoattractant as compared to control ($p < 0.05$). This novel assay technique showed 100% sensitivity and 99.21% specificity for chemotaxis assay of *Pseudomonas putida* MCC 2989 towards 3 mM L-aspartate over 50 min assay time. The device was also used to isolate bacteria chemotactic to caffeine directly from environmental samples. Very high chemotaxis response indices were reported for the first-time using chemotaxis plate.

**Keywords** Chemotaxis · *Pseudomonas putida* · *Bacillus subtilis* · L-aspartate · Caffeine · Chemoattractant

## Introduction

Chemotaxis is defined as movement of cells under the influence of a chemical gradient [1, 2]. Bacterial chemotaxis can be either metabolism dependent or independent [3], and the movement has been attributed to finding optimum growth or survival conditions [4, 5], quorum sensing, biofilm formation and several other important functions [6]. Since chemotaxis is the basis of cell signaling, development, response, and metabolic pathways, it has attracted attention of numerous research groups worldwide.

Various devices and methods have been developed to make study of chemotaxis possible. Capillary assay is one such technique, used to study bacterial chemotaxis utilizing chemoattractant in 1 µL microcapillary, kept in contact with 0.2 mL bacterial culture to evaluate chemotactic response [1]. This is a routine method with or without modifications [2, 7, 8], however, the method is very tedious [9]. In another method, 0.1 mL of bacterial suspension is taken in a micropipette tip and a syringe needle containing chemoattractant is dipped in the tip to accumulate chemotactic cells in needle [10]. In this method, chemoattractant containing capillary/needle comes in direct contact with cell suspension. Similar other methods can be found in the literature [11]. Several chemotaxis study devices based on microfluidic technology also have been previously described [12–19]. These devices are fabricated having dimensions in the range of micrometers to a few millimeters allowing the working volume to be in the range of nanoliter to microliter. This can be useful for study of limited number of cell types. Some limitations of existing devices and assemblies are described below.

Microfluidic devices have complex workflow and very limited sample volumes, that is, in nanoliters to a few microliters. However, for environmental samples, higher volumes give better representation of bacterial diversity and abundances, limiting the use of microfluidic devices. In capillary assays, use of sample volume up to 0.2 mL is described. But the capillary filled with chemoattractant (or buffer for control experiment) is placed in direct contact with cells,

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making the distance to be traversed by cells under the influence of chemical gradient, to be 0 µm. Distance of up to 18 mm is described in some microfluidic devices. This path is linear for most of the devices. This leads to the accumulation of more cells in control experiment, resulting in reduced chemotaxis response index. Furthermore, all these factors increase the probability of non-chemotactic cells with random motility, to travel to chemotaxis observation areas by chance. Majority of methods describe working with pure cultures or two to three morphotypes together in a chemically defined diluent such as a buffer. The literature on use of such devices for environmental samples is grossly inadequate [20]. Additionally, requirement of sterility of the assembly and maintenance of aseptic conditions throughout experiment is of prime importance for bacterial studies. Authors attempted to overcome these limitations by designing a novel device and method.

Present study describes an innovative chemotaxis plate that is economic as well as easy to assemble, sterilize, use and reuse. A simple method to use this device allowing usage of higher sample and assay volumes for quantitative determination of chemotactic response to a chemoattractant has been described. The assembly was used in a petri dish, autoclaved and reused. The method yielded very high chemotaxis indices emphasizing the assay specificity. It was also used to isolate caffeine chemotactic bacteria directly from environmental samples and showed promising results.

## Materials and Methods

### Cultures

Lyophilized cultures of *Pseudomonas putida* MCC 2989 and *Bacillus subtilis* MCC 2049 were purchased from National Centre for Microbial Resource (NCMR), National Centre for Cell Science (NCCS), Pune, India. These cultures were selected based on previous reports of chemotaxis towards L-aspartate [21, 22]. Cultures were revived on nutrient agar and trypticase soy agar respectively as per culture collection guidelines. Active cultures were preserved on nutrient agar at 4 °C. Long term preservation was done in glycerol stocks as well as by lyophilization. All the chemicals and reagents used for the study were purchased from HiMedia Laboratories, India.

### Swarm Plate, Drop Plate and Capillary Assay of *P. putida* and *B. subtilis*

Growth curve of both cultures was studied to identify mid-exponential phase for further experiments [1]. The chemotactic response of *P. putida* MCC 2989 and *B. subtilis* MCC 2049 to L-aspartate was confirmed using swarm plate assay [23, 24]. 1 mM L-aspartate solution was filter sterilized through 0.22 µ filter and used for swarm plate assay. Drop plate assay was performed as described previously [23, 25]. The chemotactic response of both cultures to L-aspartate was quantitated using capillary assay [1, 2] using 1 mM L-aspartate as chemoattractant in 1 µL capillaries purchased from Hirschmann Laborgerate, Germany. Chemotaxis buffer (100 mM potassium phosphate, pH 7.0, 20 µM potassium EDTA) [2] was used instead of chemoattractant in control capillaries.

### Chemotaxis Plate (CP) Assembly

The novel device henceforth referred as Chemotaxis Plate (CP) was designed in AutoCAD and molded in Teflon at a local enterprise in Pune. The device dimensions were chosen such that the CP could be placed in glass Petri dish of 90 mm diameter and 15 mm height. This allowed the assembly to be easily autoclaved and handled aseptically throughout the experiment. Indian Patent and PCT applications have been filed for protection of intellectual property concerning the device and method described in present study. Please refer to Fig. 1 for general aspects of CP design.

A thimble (diameter and height of 10 mm) made of Whatman filter paper No. 1 was placed at the central port of the device. The CP was then placed in petri dish and autoclaved at 15 psi for 20 min. For each experiment, the sterile CP assemblies were set on a vibration-free coplanar platform adjusted using level tube to maintain uniform gradient of the chemoattractant. Uniform gradient formation was confirmed using methyl red dye solution. The maximum working volume of CP was 15 mL. All four culture/ environmental sample addition points (sites 1 in Fig. 1E) were used. Assay sample collection points (sites 2 in Fig. 1E) were used to retrieve aliquots to analyze chemotactic response at regular time intervals. L-aspartate was used as a chemoattractant to validate the device using *P. putida* and *B. subtilis*. Caffeine was used as a chemoattractant for studies with environmental samples as described below.

### Chemotaxis Assay of *P. putida* and *B. subtilis* Using Chemotaxis Plate

The sterile assemblies were readied as described in the previous section 14.75 mL of chemotaxis buffer was added to CP and the assembly was allowed to stand for one min for uniform wetting of the thimble. Mid-exponential phase cultures of *P. putida* MCC 2989 and *B. subtilis* MCC 2049 were harvested by centrifugation at 8000 rpm at 25 °C. The cell pellets were washed twice with chemotaxis buffer to remove traces of the medium [1]. 37.5 µL of washed culture was added slowly to each of four sample addition points. Thus, the total amount of culture added was

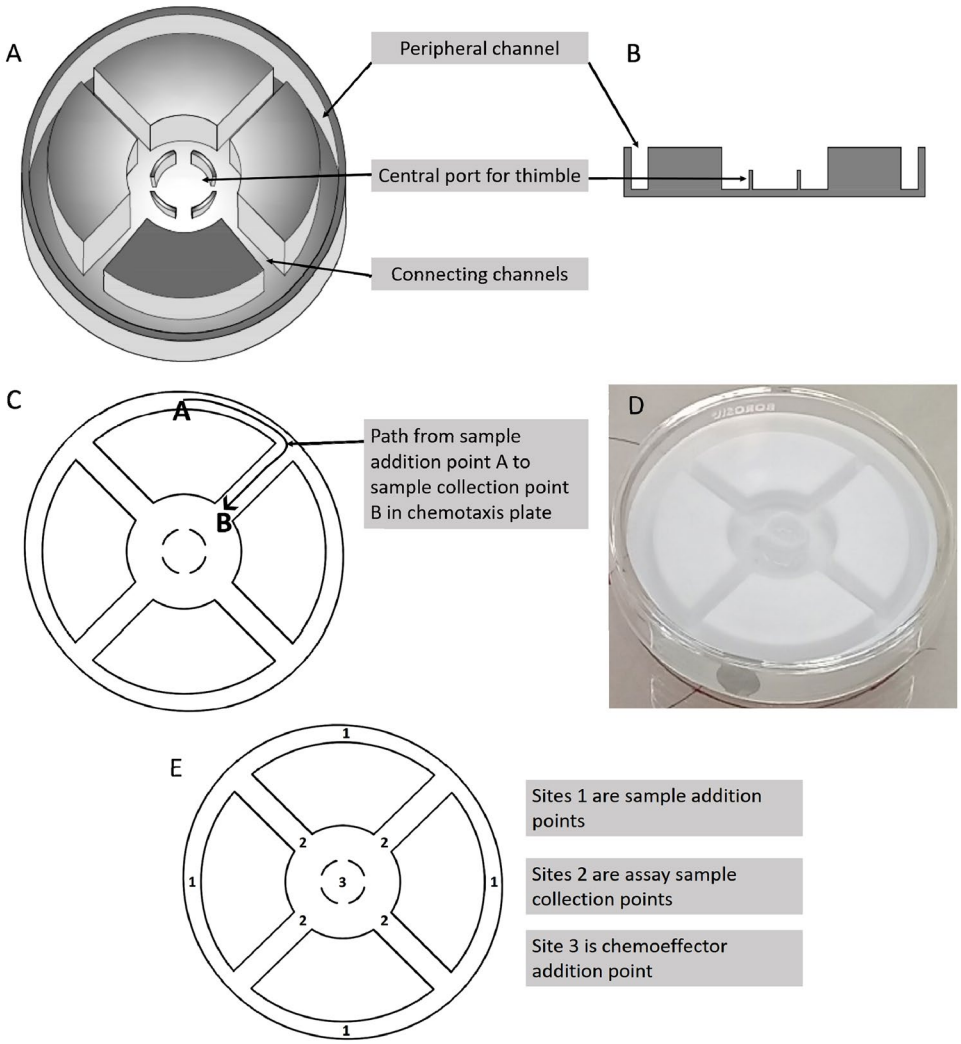


**Fig. 1** Chemotaxis plate (**A**) 3D view showing different features (**B**) Cross-sectional view (**C**) Path from sample/culture addition point A to assay sample collection point B (**D**) The actual assembly in Petri dish (**E**) Sketch showing different sites of the device

0.15 mL corresponding to cell density of ~ 1 X $10^9$ CFU/mL. This was immediately followed by addition of 0.1 mL of chemoattractant to the central thimble to create a gradient of L-aspartate. Thus, the total working volume of assay was 15 mL giving rise to 100-fold dilution of inoculum to reach to the desired cell density of ~ 1 X $10^7$ CFU/mL. This cell density was equal to that of capillary assays described earlier. Aliquots of 0.1 mL each, was removed from the sample collection point at intervals of 10 min up to 50 min. These aliquots were serially diluted and spread plated on nutrient agar. The plates were incubated for 24 h at 28 °C for *P. putida* MCC 2989 and 37 °C for *B. subtilis* MCC 2049 to obtain the required cell counts. Same protocol was followed for control experiment, where chemotaxis buffer was added to the central thimble instead of L-aspartate. All experiments were performed in triplicates. Chemotaxis response index was calculated as the ratio of number of cells recovered in test to that in control [10]. t-test was used for data analysis unless indicated otherwise. The specificity and sensitivity of assay was calculated as per formulae reported previously [26].

### Effect of L-Aspartate Concentration

Three concentrations of L-aspartate, 1 mM, 3 mM and 5 mM were used for optimization based on previous literature. 0.1 mL of each concentration was added to the central thimble to observe its effect on number of cells accumulated at the sample collection point as per the protocol described in previous section.

### Effect of Cell Density

Lower cell density results in low substrate utilization leading to more effective maintenance of gradient for chemotaxis study [9]. Thus, objective of this experiment was to check accumulation of chemotactic cells using lower initial cell concentration. 3 mM L-aspartate was found

to be most effective for chemotactic cell recovery using chemotaxis plate and thus, was chosen for this experiment. Lower cell recovery was observed for *B. subtilis* in previous experiments as compared to *P. putida* and it also required more assay time (50 min) out of the two. Hence, only *P. putida* was used for this experiment. Inoculum concentration of 5 X $10^6$ CFU/mL was used, which is half of previous experiments.

### Chemotaxis of Mixed Population in Environmental Sample Using Chemotaxis Plate

River water and sediment samples were collected from domestic wastewater discharge site in Mutha River, Pune, India, with the coordinates 18°30′28.7"N, 73°50′20.1"E. Samples collected in sterile sample collection containers were immediately transported and processed in laboratory. Physico-chemical properties such as color, pH and optical density were recorded for both the samples. Sediment sample was homogenized by shaking at 100 rpm for 30 min before processing.

Total viable count of both samples was obtained on nutrient agar. Water sample was directly used for chemotaxis assay using caffeine as chemoattractant. Homogenized sediment sample was centrifuged to settle solids and supernatant was used for chemotaxis assay. Protocol described in previous section was used for chemotaxis assay of samples using CP with 3 mM caffeine as chemoattractants. Control experiments were run using chemotaxis buffer instead of chemoattractant. Capillary assays were performed for both samples along with CP assay. Simultaneously, the samples were inoculated in M9 minimal broth and agar (components in g/L: 15.0 $Na_2HPO_4.12H_2O$, 3.0 $KH_2PO_4$, 0.5 NaCl, 0.25 $MgSO_4$, 1.0 $NH_4Cl$) containing 0.1% (w/v) caffeine as sole carbon source [27] and incubated at 28 °C at 90 rpm for 72 h for enrichment of caffeine degraders. The enrichment cultures were also used for chemotaxis assay using CP. Isolates obtained using CP assays were purified and preserved until further use.

### Swarm Plate Assay of Bacteria Isolated Using Chemotaxis Plate

Colony characters were used to discern the isolates as different morphotypes. The chemotaxis of all isolates towards caffeine was confirmed using swarm plate assay in triplicates using 1 mM caffeine as chemoattractant. All assay plates were incubated at 28 °C and results were noted after 24 to 48 h incubation.

## Results and Discussion

### Swarm Plate, Drop Plate and Capillary Assay of *P. putida* and *B. subtilis*

*P. putida* MCC 2989 and *B. subtilis* MCC 2049 exhibited chemotaxis to L-aspartate confirmed by swarming growth in rings on swarm and drop assay plates (Fig. 2). These results are consistent with previous reports [21, 22].

Figure 3 denotes the results obtained through capillary assays. The error bars in figures indicate standard deviation observed in colony counts. For *P. putida* MCC 2989, $173 \pm 0.71$ X $10^3$ CFU/mL accumulated at 40 min interval in test capillary containing 1 mM L-aspartate as compared to 1 X $10^3$ CFU/mL in control capillary containing chemotaxis buffer. The counts for *B. subtilis* MCC 2049 were $7.05 \pm 1.34$ X $10^3$ CFU/mL for test and zero cell recovery at 50 min for control indicating chemotaxis of both cultures to L-aspartate.

### Chemotaxis Assay of *P. putida* and *B. subtilis* to L-Aspartate Using Chemotaxis Plate

*Effect of L-Aspartate Concentration*

The counts of bacteria recovered in control and test experiments are represented in Fig. 4. There were no to very few bacteria recovered in 1 mM L-aspartate test as well as

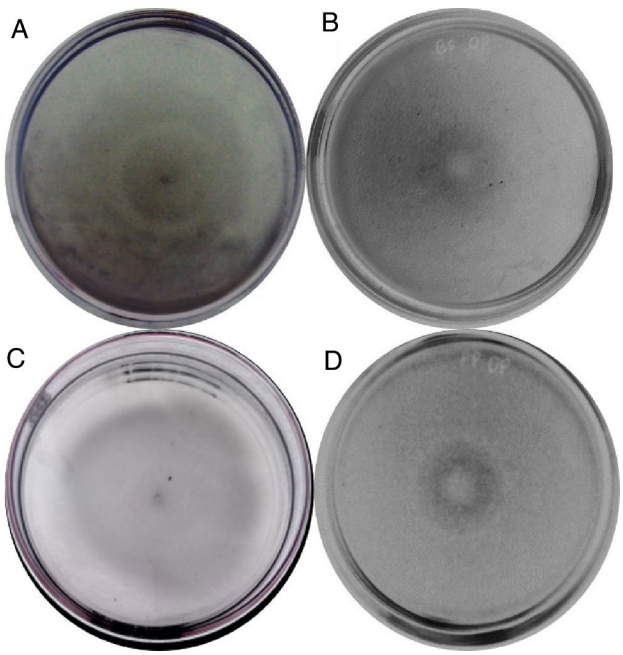


**Fig. 2** Swarm and drop plate assay using 1 mM L-aspartate as chemoattractant. *B. subtilis* MCC 2049 (**A**) swarm plate (**B**) drop plate. *P. putida* MCC 2989 (**C**) swarm plate (**D**) drop plate

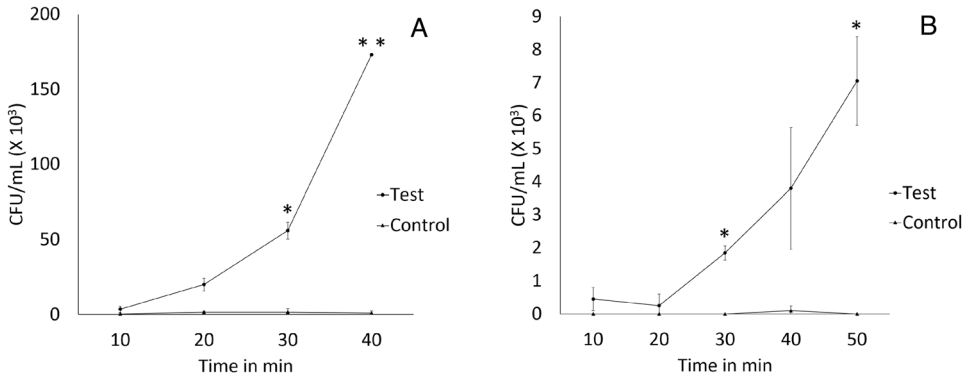


**Fig. 3** Cell counts observed in capillary assay using 1 mM L-aspartate as chemoattractant (**A**) *P. putida* MCC 2989 (**B**) *B. subtilis* MCC 2049. Statistical significance *($p < 0.05$), **($p < 0.01$) calculated using t test

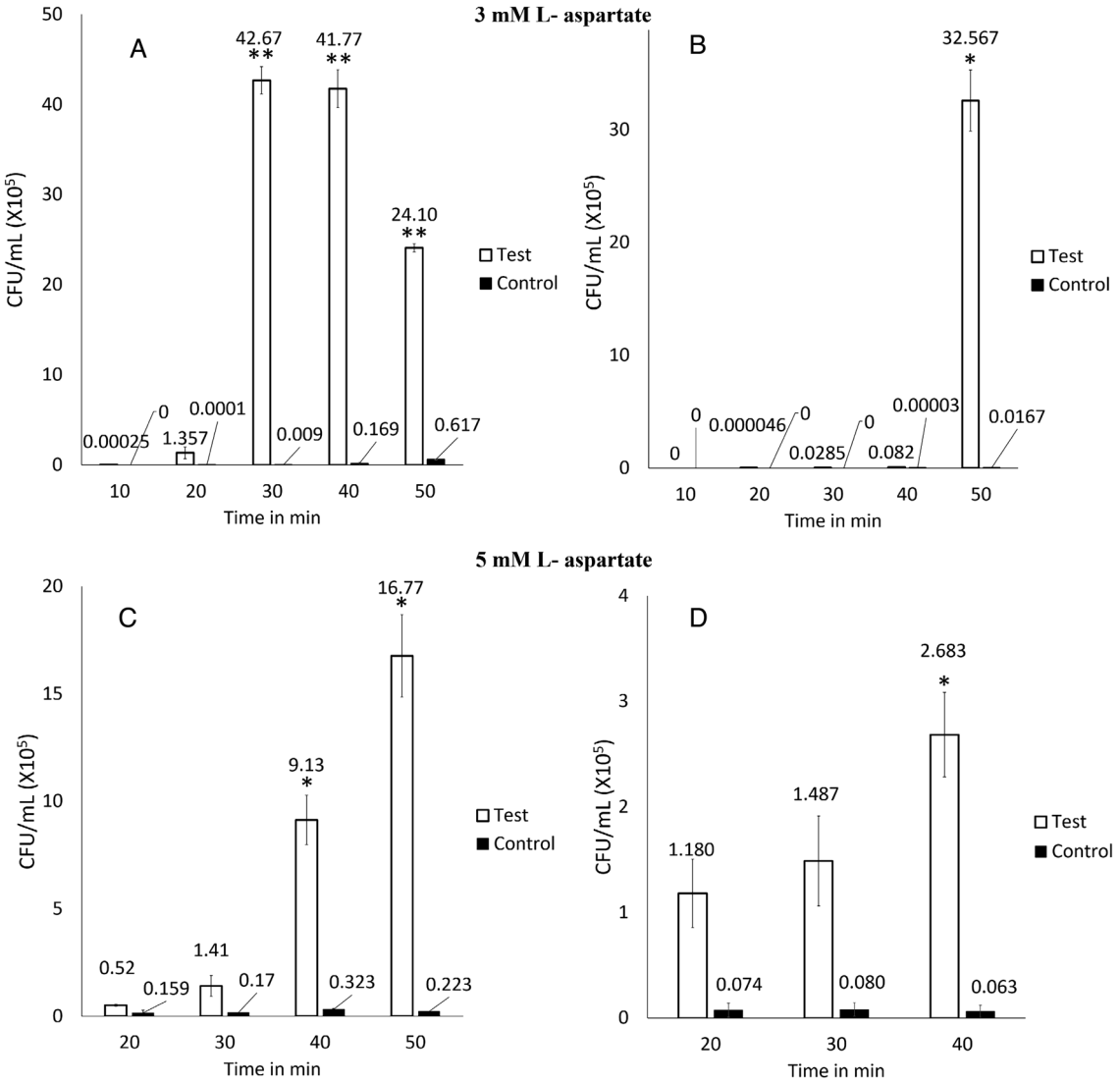


**Fig. 4** Cell counts observed by chemotaxis plate assay of (**A**, **C**) *P. putida* MCC 2989 and (**B**, **D**) *B. subtilis* MCC 2049 using 3 and 5 mM L-aspartate as chemoattractant respectively. Significant difference in test and control counts is indicated as * for ($p < 0.05$) and ** for ($p < 0.01$)

control assay and thus were not included in further analysis. $42.67 \pm 1.53$ X $10^5$ CFU/mL of *P. putida* were recovered in presence of chemoattractant (3 mM L-aspartate) as compared to $9 \pm 8$ X $10^2$ CFU/mL accumulated in absence of chemoattractant at 30 min (Fig. 4A). The cumulative accumulation of *P. putida* cells over the period of 50 min was 1 X $10^7$ CFU/mL which was equal to the inoculum size indicating 100% sensitivity and 99% specificity at 3 mM L-aspartate concentration. $32.57 \pm 2.7$ X $10^5$ CFU/mL of *B. subtilis* were recovered in test and $16.7 \pm 4.9$ X $10^2$ CFU/mL in control at 50 min. Overall 99.98% specificity was observed for cumulative cell accumulation (Fig. 4B). Test cell recovery was found to be significantly higher ($p < 0.01$, 0.05) than control.

At 5 mM L-aspartate concentration, $1.67 \pm 0.191$ X $10^6$ CFU/mL of *P. putida* were recovered at 50 min in test which was significantly higher ($p < 0.05$) as compared to $2.23 \pm 0.117$ X $10^4$ CFU/mL recovered in control (Fig. 4C). For *B. subtilis*, $2.68 \pm 0.4$ X $10^5$ CFU/mL were recovered in test at 40 min which was significantly higher ($p < 0.05$) compared to $6.27 \pm 5.75$ X $10^3$ CFU/mL recovered in control (Fig. 4D). The number of cells recovered using 3 mM L-aspartate concentration were significantly higher than those recovered using 5 mM concentration for both *P. putida* and *B. subtilis* (one way analysis of variance, $p < 0.05$). This may be due to the gradient shape [28] and location of assay sample collection point. At 3 mM concentration of chemoattractant, the optimum concentration of L-aspartate in the gradient must be present at the assay sample collection point, leading to higher cell recovery. However, at a higher concentration of 5 mM, the point of optimum concentration of L-aspartate might be farther from the assay sample collection point leading to lower cell recovery. In such cases, a site more distant from chemoattractant addition point may yield higher cell count and can be decided based on trial experiments. This reason may as well underlie the sudden rise in cell recovery observed in 3 mM chemoattractant concentration. The optimum concentration of L-aspartate may be at sample collection point where high number of cells were attracted leading to sudden rise in number. In contrast, a gradual rise in cell recovery was observed in 5 mM concentration. This may be due to metabolism dependent chemotaxis where L-aspartate might have been utilized by bacteria changing the gradient shape and shifting the optimum concentration slowly towards assay sample collection point leading to gradual increase in count.

Further, the quantitative data obtained in present study was compared with previous reports. 5 X $10^5$ CFU/mL of *E. coli* accumulated in capillary with 1 mM L-aspartate [1], whereas, tenfold higher CFU/mL were recovered using chemotaxis plate assay for both *P. putida* and *B. subtilis*. Bacterial colony counts reported using other chemotaxis assays are summarized in Table 1. The chemotactic colony counts observed in the present study using chemotaxis plate are consistent with some and higher than most of the previous reports.

### *Effect of Cell Density*

At the cell density of 5 X $10^6$ CFU/mL of *P. putida*, $39.05 \pm 3.18$ X $10^5$ CFU/mL were recovered in test as compared to $6 \pm 1.1$ X $10^2$ CFU/mL in control at 40 min.

**Table 1** Previous reports on bacterial chemotactic cell recovery and chemotaxis response indices using various assays

| Organism | Chemoattractant used | Assay used | Count (CFU/mL) or CI | References |
|---|---|---|---|---|
| *P. putida* MCC 2989 | 3 mM aspartate | Chemotaxis plate | $42.67 \pm 1.53$ X $10^5$ CI 4615.38 | Present study |
| *B. subtilis* MCC 2049 | 3 mM aspartate | Chemotaxis plate | $32.57 \pm 2.7$ X $10^5$ CI 2096.77 | Present study |
| *E. coli* | 0.1 mM aspartate | Microfluidic chip | > 600 cells | [18] |
| *P. putida* G7 | 1 mM aspartate | Modified capillary | 3 X $10^2$ | [7] |
| *Pseudomonas* strain ADP | 0.02 mM cyanuric acid | Capillary | ~60 X $10^3$ | [29] |
| *Pseudomonas* sp. B4 | 1 mM biphenyl | Modified capillary | 13 X $10^4$, CI > 2 | [30] |
| *P. putida* F1 | 1 mM toluene | Capillary | 2 X $10^5$ | [31] |
| *E. coli* | 1 mM L-asp | Capillary | 5 X $10^5$ | [1] |
| *B. amyloliquefaciens** | root exudates of banana | Modified capillary | ~70 X $10^5$ | [32] |
| *P. putida* strain PKJ15 | 2 mM salicylate | Capillary | CI 5.8 | [25] |
| *Ralstonia* sp.SJ98 | 0.2 mM aspartate | Capillary | CI 16 | [8] |
| Natural sample | 1 mM glutamine | Novel in situ | CI 18 | [20] |
| *Pseudomonas* sp. BUR11 | 350 ppm citrate | Capillary | CI 40 | [33] |
| *Pseudomonas* sp. JHN | 550 µM aspartate | Capillary | CI 60 | [34] |

*Initial O. D. = 0.8, high recovery in control ~ 35 X $10^5$ CFU/mL

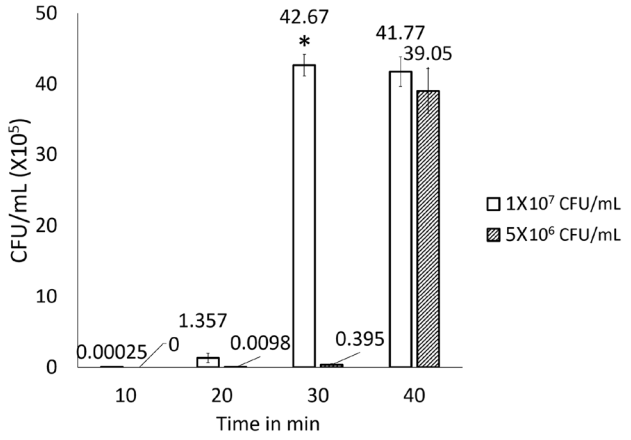


**Fig. 5** Comparison of counts of *P. putida* at two initial cell densities using 3 mM L-aspartate. Significant results are indicated by * ($p < 0.05$)

Figure 5 shows that initial cell density of 1 X $10^7$ CFU/mL gives significantly better results at 30 min assay time ($p < 0.05$). However, the counts equalized with the increase in assay time.

In a report on use of low cell density, 2 X $10^3$ CFU/mL of *P. putida* G7 accumulated in continuous flow capillary assay using naphthalene as chemoattractant with initial cell density of $10^5$ cells [9]. In present study 39.05 X $10^5$ CFU/mL of *P. putida* were recovered with initial cell density of 5 X $10^6$ CFU/mL.

## Chemotaxis Assay of Mixed Population in Environmental Sample Using Chemotaxis Plate

### *Direct Sample Assay*

The water sample was visually clear with pH 7.6 and TVC of 1.3 X $10^6$ CFU/mL having 36 bacterial colony morphotypes on nutrient agar and 17 morphotypes including one fungal colony on caffeine agar. The sediment sample color was black, pH 8 and TVC of 2.9 X $10^7$ CFU/mL having 41 bacterial colony morphotypes on nutrient agar and 15 morphotypes on caffeine agar. Growth on M9 minimal agar containing 0.1% caffeine as sole carbon source confirmed presence of caffeine degraders in both samples.

The capillary assay count at 30 min with 1 mM caffeine as chemoattractant was found to be 1.02 X $10^3$ CFU/mL for test and 0.85 X $10^3$ CFU/mL for control for water sample. This indicated presence of bacteria chemotactic to caffeine in water sample. With CP assay of water sample, 3.5 X $10^3$ CFU/mL were obtained in test experiment using caffeine chemoattractant, whereas, 2.9 X $10^2$ CFU/mL in control experiment in absence of caffeine. Considerably higher number of cells were recovered in CP method than capillary assay. The test count using 3 mM caffeine as chemoattractant was consistently higher as compared to control count for both samples. Seven chemotactic bacterial colony morphotypes were obtained after CP assay. For sediment sample, the number of cells recovered in test and control experiment were similar in both capillary and CP assay.

### *Enrichment Culture Assay*

The TVC of water sample caffeine enrichment culture was 1.53 X $10^8$ CFU/mL comprising nine morphotypes and that of sediment sample caffeine enrichment culture was 1.22 X $10^9$ CFU/mL with eight morphotypes on nutrient agar. The result obtained for enrichment culture CP assay with 3 mM caffeine as chemoattractant at 20 min, for water sample enrichment culture was $3.05 \pm 0.35$ X $10^4$ CFU/mL which is tenfold higher as compared to 4 X $10^3$ CFU/mL recovered in capillary assay. For sediment sample, CP assay count was $4.92 \pm 0.141$ X $10^5$ CFU/mL which was 100 fold higher than capillary assay count of 4.8 X $10^3$ CFU/mL. In case of capillary assay, test and control counts were similar. On the contrary, the counts of test and control CP experiments were significantly different ($p < 0.05$) for both samples. Four chemotactic morphotypes for water sample enrichment culture and three chemotactic morphotypes for sediment sample enrichment culture were observed on CP assay plates. Swarm plate assay was performed to confirm chemotaxis of each isolate towards caffeine. Table 2 summarizes the

**Table 2** Colony counts and morphotypes obtained at different stages of sample processing

| | Sample[a] | | | | Enrichment culture[a] | | |
|---|---|---|---|---|---|---|---|
| | TVC | Morphotypes on caffeine agar | CP assay count | Capillary assay | TVC | CP assay count | Capillary assay |
| Water sample | 1.3 X $10^6$ (36) | 17 | 3.5 X $10^3$ (7) | 1.02 X $10^3$ (5) | 1.53 X $10^8$ (9) | 3.05 X $10^4$ (4) | 4 X $10^3$ (4) |
| Sediment Sample | 2.9 X $10^7$ (41) | 15 | 4.8 X $10^3$ (5) | 2.01 X $10^3$ (4) | 1.22 X $10^9$ (8) | 4.92 X $10^5$ (3) | 4.8 X $10^3$ (3) |

[a]Number of morphotypes are indicated in parentheses

observations of colony counts and morphotypes recorded at each step.

*Swarm Plate Assay of Environmental Isolates*

Seven isolates (four from water and three from sediment sample enrichment culture) obtained using CP were found to be highly motile and exhibited chemotaxis towards caffeine in swarm plate assay. Figure 6 depicts the results of swarm plate assay. The isolates were further screened and studied for caffeine degradation; however, the data of these experiments is beyond the scope of this article.

There is paucity of studies on accumulation of chemotactic cells directly from environmental samples using chemotaxis devices. One study reported enrichment of bacteria chemotactic to benzoate directly from environmental sample using SlipChip where 1.95 X $10^2$ cells *C. testosteroni* CNB-1 were obtained with benzoate as chemoattractant [19]. In present study, ~ 18 fold more, i.e., 3.5 X $10^3$ CFU/mL of chemotactic cells were recovered directly from water sample using chemotaxis plate and 3 mM caffeine as chemoattractant.

*Chemotaxis Response Indices (CI)*

The chemotaxis response index (CI) was calculated as described earlier [10]. The CI for time interval with highest cell accumulation in test was calculated for both capillary and CP assays. High chemotaxis response indices were obtained with CP assay due to negligible cell recovery in absence of chemoattractant. Capillary assay performed using 1 mM L-aspartate showed CI of 38 and 173 at 40 min interval for *B. subtilis* MCC 2049 and *P. putida* MCC 2989 respectively. Whereas CI of 2096.77 at 50 min and 4615.38 at 30 min interval was recorded for *B. subtilis* MCC 2049 and *P. putida* MCC 2989 respectively using CP assay with 3 mM L-aspartate. The CI of *P. putida* was higher requiring less time as compared to *B. subtilis*. The plausible reason may be the innate difference in the strength of L-aspartate as chemoattractant for these two organisms. As per literature, bacterial chemotaxis responses depend on several factors like the mechanism of motility, chemoreceptors and chemotaxis pathway. The chemoattractant can be strong or weak for different types of organisms which decides the rate of chemotaxis. In this study, *P. putida* and *B. subtilis,* having different Gram character, flagellation type and chemotaxis pathways [28, 35, 36], may have responded with different degree of chemotaxis towards L-aspartate. *P. putida* might be exhibiting stronger chemotaxis to L-aspartate than *B. subtilis* leading to higher CI.

The sample chemotaxis indices obtained using capillary assay are, 1.2 for water sample, 9.52 for water sample enrichment culture and 15 for sediment sample enrichment culture at 30 min interval. CP assay showed CI of 12.17 for water sample at 50 min interval and 49,200 for sediment sample enrichment culture at 20 min interval which is the highest index obtained in this study. Sediment samples showed CI of 1 in both assays. CI of water sample enrichment culture could not be determined as zero cells were

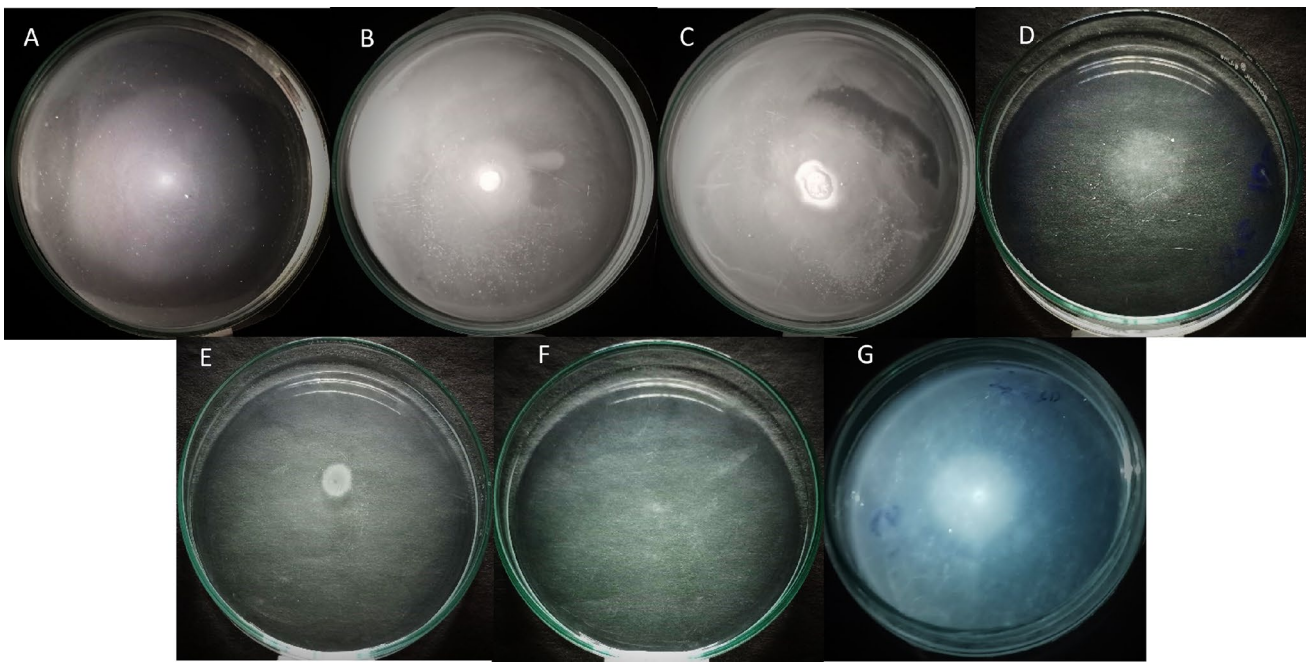


**Fig. 6** Swarm plate assay of environmental isolates using caffeine as chemoattractant

recovered in control of CP assay. This is the first study to report very high CI values for bacterial chemotaxis indicating specificity of the device.

Table 1 summarizes chemotaxis indices reported previously. Chemotaxis indices obtained using CP assay are higher than any of the previous reports for bacterial chemotaxis. This is because of negligible cell accumulation in absence of chemoattractant in control experiments. This can be the major advantage of CP assay increasing the significance and reliability of assay. Negligible cell recovery in control can be explained with the help of device dimensions. The distance between cell addition to sample collection point is ~ 65 mm (Fig. 1C, point A to B) making it difficult for cells with random movement to reach the sample collection point within the assay time. Moreover, the path from cell addition to sample collection point is not linear (Fig. 1C), decreasing the probability of cell recovery in control experiment even further. Moreover, previous designs have zero to up to 18 mm distance between cell addition and chemoattractant addition points. In the device described in present study, this distance was 65 mm that is 3.6 to 100-fold more than previously described devices which aided in low accumulation in control resulting in high CI.

The prime function of any chemotaxis device used for positive chemotaxis study is allowing accumulation of chemotactic cells under the influence of chemoattractant and either qualitative or quantitative evaluation of chemotaxis response minimizing false positive results. The device described in present study allowed quantitative evaluation of bacterial chemotaxis response. *P. putida* and *B. subtilis* are previously reported to be chemotactic to L-aspartate and hence were used in this study. In present study, authors reported 100% sensitivity and 99.21% specificity indicating very low false positive result for *P. putida* with 3 mM L-aspartate concentration. The specificity for *B. subtilis* was also very high, i.e. 99.98% but sensitivity was low as only 3.26 X $10^6$ cells accumulated out the 1 X $10^7$ cells in inoculum. However, sensitivity can be further improved by optimizing the process for *B. subtilis.* Very high chemotaxis indices were reported for the first time due to low cell recovery in control emphasizing the specificity of device. However, the present study is limited to use of new device for the study of chemotaxis towards chemoattractants and cannot be used to study volatile chemo-effectors, chemo-repellents, aerotaxis, thermotaxis or other movements without modifications in device and method. The inoculum used for experiments of chemotaxis plate was based on the densities used in previous reports. In present study, the highest density of culture was $10^9$ CFU/mL and lowest effective density was of $10^6$ CFU/mL indicating that the device can be conveniently used in this range. Although the present study used bacteria for chemotaxis assay, its use may be extended to other organisms belonging to unicellular, freely motile algae, yeasts and slime molds, however, it may be of little use for filamentous fungi.

## Conclusion

The chemotaxis plate described in present study provides a cost-effective, reliable, easy to assemble & sterilize, and reusable alternative to previously described bacterial chemotaxis assay devices. Chemotaxis plate allows use of larger volumes beneficial for direct assay of environmental samples. Larger volume may also allow dilution of chemotaxis inhibitors, if any, present in the samples. This assay yielded 100-to-1000-fold rise in cell recovery and first report on very high CI as compared to previous reports on bacterial chemotaxis. Nevertheless, more studies with different types of chemoattractants, type cultures such as *E. coli*, other cultures such as algae, slime molds, yeasts and environmental samples are required to evaluate and expand the applicability of this device.

### Declarations

**Competing interests** The authors declare that they have no known competing financial or non-financial interests that could have appeared to influence the work reported in this paper.

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