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Decoding the Chemical Language of Motile Bacteria by Using High-Throughput Microfluidic Assays

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Abstract

Motile bacteria navigate chemical environments by using chemoreceptors. The output of these protein sensors is linked to motility machinery and enables bacteria to follow chemical gradients. Understanding the chemical specificity of different families of chemoreceptors is essential for predicting and controlling bacterial behavior in ecological niches, including symbiotic and pathogenic interactions with plants and mammals. The identification of chemical(s) recognized by specific families of receptors is limited by the low throughput and complexity of chemotaxis assays. To address this challenge, we developed a microfluidic-based chemotaxis assay that is quantitative, simple, and enables high-throughput measurements of bacterial response to different chemicals. Using the model bacterium *Escherichia coli*, we demonstrated a strategy for identifying molecules that activate chemoreceptors from a diverse compound library and for determining how global behavioral strategies are tuned to chemical environments.

Keywords

chemoreceptors; chemotaxis; high-throughput assays; microfluidics

Motile bacteria have evolved a sophisticated strategy, referred to as chemotaxis, for navigating gradients of small molecules and ions. Chemotaxis enables motile bacteria to find nutrients, evade harmful compounds, and identify locations for growth and community formation. Pathogenic and symbiotic bacteria adapt to host microenvironments by using chemotaxis, and recent studies have demonstrated that deficiencies in chemotaxis can impair pathogenesis.^[1] The collective migration of chemotactic bacteria facilitates large-scale ecological processes, including carbon cycling in oceans.^[2] Chemotaxis affects a wide range

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of bacterial processes and provides a unique pharmacological target for modifying bacterial behavior and altering pathogenic phenotypes.^[3]

Bacteria use sensitive chemical sensors, referred to as chemoreceptors, to monitor extracellular gradients of ions and chemicals. Different families of chemoreceptor proteins define the chemical vocabulary of bacteria. Genome sequencing and bioinformatics suggest that each motile bacterial species contains 10–20 different chemoreceptor types that each recognize a set of structurally related molecules.^[4] Molecular biology, biochemistry, and biophysics revealed the molecular mechanisms underlying the chemotaxis systems; however, the chemoreceptors in most bacterial species have not been chemically characterized. The absence of a general census of cognate chemical/chemoreceptor pairs makes it extremely difficult to predict bacterial behavior in chemical environments.

A fundamental barrier to assigning chemical/receptor pairs is the low throughput of current bacterial chemotaxis assays. The capillary assay is the gold standard for measuring chemotaxis;^[5] however, these assays require considerable finesse to achieve reproducibility and are generally serial and not designed for multiplexing. Other techniques have emerged more recently,^[6] including several based on microfluidics that are designed to increase the sensitivity of chemotaxis measurements and enable users to control experimental parameters, such as the shape of chemical gradients.^[7] A recent paper described chemotaxis assays to screen a library of 80 compounds for antagonists of a known *Escherichia coli* chemoreceptor.^[3]

We report a simple system for performing an arbitrarily large number of parallel chemotaxis assays. Our approach incorporates a passive architecture to fill microfluidic channels, generate stable chemical gradients rapidly, and quantify assay output quickly and easily. These assays can be performed without specialized external equipment, are scalable, and are accessible to non-expert end users. Using the model chemotactic bacterium *E. coli*, we characterized the sensitivity of the device and performed a small-scale chemical–genetic screen that demonstrated how a high-throughput approach could facilitate the identification of chemical/receptor pairs. Furthermore, we demonstrated that global chemotaxis strategies are tuned to the presence of specific environmental chemicals.

Our parallel chemotaxis assay is illustrated in Figure 1. For simplicity, we used a single microfluidic design for all of the chemotaxis experiments in this communication. This radial design enables 45 separate compounds to be assayed in a single experiment. In our design, 45 identical microchannels (50 μm tall and 100 μm wide) emanate from a central inlet (Figure 1A), all of which are embossed into a layer of poly(dimethylsiloxane) (PDMS). Each channel terminates with two circular chambers. We refer to the larger chamber (300 μm tall and 3 mm diameter) as the pool, and the smaller, terminal chamber (50 μm tall and 2 mm diameter) as the source (Figure 1E). As detailed below, our assay generates a stable chemical gradient that extends from the source to the pool. Chemotactic responses to the chemical gradients are quantified by measuring the accumulation of bacteria in the source chamber. Further details describing the construction and operation of the assay are provided in the Supporting Information.

To assemble the assay, we added 1 μL droplets of compound solutions to each source chamber (Figure 1A). After drying, we inverted the PDMS layer and brought it into conformal contact with a glass or plastic slide to seal the microfluidic channels (Figure 1B).

The microfluidic channels were filled with a dilute bacterial suspension by using a passive pumping method referred to as degas-driven flow.^[8] Briefly, degas-driven flow leverages the gas permeability of the polymer used to construct the microfluidic network (here, PDMS) and is particularly useful for filling dead-end channels, such as those in our design. We placed the fully assembled assays in a vacuum for 90 min to reduce the amount of gas dissolved in the PDMS. After removing the assay from the vacuum, we placed a 120 μL drop of a dilute bacterial suspension over the central inlet (Figure 1C). As the PDMS returned to atmospheric pressure, the PDMS walls of the microfluidic channels absorbed the trapped air in the microfluidic network and drew the bacterial suspension into the microchannels (Figure 1C and D). Degas-driven flow filled the entire channel network uniformly in ~ 20 min. To limit evaporation of the bacterial solution at the inlet, we placed a drop of mineral oil on top of the water (Figure 1C). Once filled, the chemicals in the source chambers dissolved and diffused toward the pool.

To generate stable chemical gradients, we took advantage of permeation-driven flow. PDMS is slightly water-permeable, and the absorption of water into the walls of microchannels generates predictable fluid flows.^[9] This subtle permeation-driven flow opposes the diffusion of chemicals from the source chambers, and equilibration of these two opposing flows produces a stable chemical gradient (Figure 1E). The formation of a stable gradient can be understood by considering how the contributions of permeation-driven flow versus diffusion change with the distance from the source chamber. At small distances from the source, the flux of chemicals due to diffusion is large while permeation-driven flow approaches zero; thus, chemicals are driven out of the source by diffusion at short distances. However, permeation-driven flow increases with the distance from the source while the flux due to diffusion decays. Thus, at greater distances, permeation-driven flow dominates and prevents the chemical gradient from diffusing ad infinitum. The equilibration of these two effects generates stable gradients with characteristic lengths on the order of a millimeter for small molecules.^[9] To confirm this mechanism of gradient formation, we monitored the formation of fluorescein gradients and their stability over time (Figure S1 in the Supporting Information). Gradients were stable after ~ 120 min and remained stable for >12 h (Figure S1). By tracking the velocity of fluorescent particles (3 μm diameter) in our design, we confirmed that permeation-driven flow travels from the inlet toward the dead end at a velocity that is >100 times slower than the swimming velocity of motile bacteria. A simple 1D diffusion–advection model was in close agreement with our empirical observations of chemical gradient formation (Figure S1), demonstrating that permeation-driven flow and molecular diffusion are the dominant forces contributing to stable gradient formation. Full descriptions of our fluorescein measurements, particle tracking, and a theoretical model are provided in the Supporting Information.

Our chemotaxis experiments focused on the model bacterium *E. coli*, as its chemotaxis system has been well-characterized, and the chemical specificity of each chemoreceptor is known. *E. coli* contains four canonical chemoreceptors—Tar (aspartate sensing), Tsr

(serine), Trg (ribose, galactose), and Tap (dipeptides)—as well as an additional chemoreceptor (Aer) that acts together with Tsr to perform “energy taxis”, which is a general response for sensing oxygen, redox levels, and other small molecule metabolites.^[10] These chemoreceptors are sensitive (capable of detecting nanomolar concentrations) and operate over a concentration range that can extend over five orders of magnitude.^[11] Our assay accurately measures chemotaxis over this entire dynamic range (Figure 2).

When the device was initially filled, bacteria were uniformly distributed at a low concentration ($\lambda = 600$ nm, absorbance = 0.04) throughout the microfluidic channels. As the chemical gradients formed, bacteria responded to attractants by swimming up gradients and accumulating in the source chambers. We counted the number of bacteria that accumulated in the source chambers by using microscopy to quantitatively measure bacterial chemotaxis. To acquire these measurements, we used *E. coli* strains that constitutively expressed the fluorescent protein dTomato (details regarding strain construction and growth conditions are provided in the Supporting Information). Three hours after the channels were filled, we imaged the fluorescent signal from the source chambers by using an epifluorescence microscope. Using fluorescently labeled bacteria increased our signal-to-noise ratio; however, unlabeled bacteria can also be measured by using a bright-field microscope with phase optics, or by the opacity of the source chamber. Figure 2A shows the fluorescence intensity from a source chamber containing an attractant (100 μ M aspartate) compared to an empty control chamber. To quantify the chemotactic response, we divide the integrated intensity of an attractant source (I_x) by the integrated intensity of an empty source (I_0) to provide relative response values (I_x/I_0) that were consistent between assays. Further details are provided in the Supporting Information.

Using relative response measurements, we acquired concentration–response curves for 27 different chemicals. Figure 2B shows concentration–response curves of wild-type *E. coli* to several canonical attractants recognized by different chemoreceptors (curves for all additional chemicals that gave measureable responses are shown in Figure S2). For each chemical tested, our measurements of half maximal effective concentration (EC_{50}) and peak response were consistent with previously reported values acquired in capillary assays (Table 1).^[12] In contrast to the capillary assay, we acquired quantitative data for dozens of chemoattractants rapidly and in parallel and found the assay to be remarkably reproducible (see standard error of the mean in Figures 2–4).

To demonstrate how high-throughput chemotaxis assays enable the assignment of the chemical specificity of uncharacterized chemoreceptors, we performed a small-scale screen to identify the chemical specificities of the chemoreceptors in *E. coli*. We established a panel of eight chemicals that included attractants recognized by each chemoreceptor in *E. coli*, integrated it into one device, and simultaneously assayed each chemical at its peak response concentration (Figure 3A). We constructed single gene deletions for all five chemoreceptors in *E. coli* (*tar*, *tsr*, *trg*, *tap*, and *aer*) from the parental strain, MG1655 (construction of gene deletions is described in the Supporting Information). Figure 3B shows the response of each deletion strain to the small molecule panel; the specificity of each chemoreceptor is represented by the lack of a response to the chemicals that are specifically recognized by the deleted chemoreceptor. Although the specificities of the

chemoreceptors in *E. coli* are established, our experiments provide a proof-of-principle for how high-throughput assays can be used to increase the pace at which new chemical/receptor pairs are discovered for the majority of motile bacteria that have uncharacterized chemotaxis systems.

In addition to assigning chemical/receptor pairs, high-throughput chemotaxis studies can reveal how bacteria optimize their global chemotaxis strategies based on changing environmental inputs. Several reports have shown that culturing conditions can have profound effects on chemotactic responses.^[13] We found that changing chemical environments alters the *E. coli* response to the carbon source provided during growth and toward other compounds (Figure 4). Interestingly, when grown in the presence of galactose, *E. coli* had an increased response to every added canonical chemoeffector. Conversely, when grown in the presence of serine, all responses were repressed. This reduced chemotactic response extended to bacterial colonies grown on low percentage agar that was supplemented with serine (Figure S3). Based on these results, we believe our assay can be a valuable tool for understanding how complex environmental signals are integrated into a global chemotactic response. Importantly, these experiments highlight capabilities of this high-throughput chemotaxis assay that would be challenging to achieve in standard chemotaxis assays.

We describe a simple, quantitative microfluidic assay for performing high-throughput measurements of bacterial chemotaxis and characterizing cognate chemical/receptor pairs. In our experiments, we describe a radial device that performs 45 parallel chemotaxis assays, but the system can be redesigned to perform an arbitrary number of assays with different layouts. As a proof-of-principle, we demonstrated a chemical-genetics screening strategy to identify chemical/receptor pairs in *E. coli*. Our future efforts are aimed at expanding this chemical screening approach to investigate uncharacterized chemoreceptors in diverse bacterial species. We found that this assay can be used to investigate how bacteria regulate their global chemotaxis strategy in response to changing chemical conditions. Most chemotaxis measurements are performed by using cells grown under standardized conditions. Our results demonstrate that *E. coli* adjusts its global chemotactic responses in response to the nutrient source used for its growth. This result is interesting, in light of bacterial mechanisms of adaptation and survival in fluctuating chemical environments. Monitoring of global chemotactic responses was enabled by the high throughput of our chemotaxis assay and is an exciting area for future investigations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) To assemble the assay, each chemical of interest was spotted in a source chamber located at the periphery of the design. B) Once the spots dried, the PDMS layer was inverted and brought into conformal contact with bare glass to seal the microfluidic channels. After the PDMS was degassed under vacuum, a dilute bacterial solution was placed over the inlet and passively filled the assay by degas-driven flow. C) Mineral oil was layered over the inlet to minimize evaporation. D) The channels filled completely in ~20 min. E) Once filled, a stable chemical gradient formed between the source and the pool over 90 min, due to a balance of diffusion and permeation-driven flow.

Figure 2.

A) Fluorescence micrographs of *E. coli* MG1655 cells constitutively expressing the fluorescent protein dTomato in a source containing an attractant (100 μ M aspartate) compared to an empty source. The dashed lines mark the edges of the source chambers. B) Chemotactic response curves toward several canonical chemoattractants are plotted as a function of chemoattractant concentration. Here, relative response represents the sum intensity of a source containing a chemical of interest (I_x) over the sum intensity of an empty source (I_0). α -Methyl-D, L-aspartate (α MA) and 2-aminoisobutyrate (AiBu) are non-metabolizable analogues of Asp and Ser that are specifically recognized by Tar and Tsr, respectively. Each value represents the mean of three measurements ($n = 3$) acquired on separate devices, and error bars indicate the standard error of the mean (SEM).

Figure 3.

A) Layout of the chemicals that were tested against each chemoreceptor deletion *E. coli* MG1655 mutant. Chemicals with matching colors were recognized specifically by the same chemoreceptor. The concentrations used were derived from the peak response values in Table 1. B) Responses of each chemoreceptor deletion strain to the panel of chemicals shown in Figure 3A. Here, the relative response values of the chemoreceptor deletion strains (R_{Δ}) were normalized to the relative responses of wild-type *E. coli* MG1655 (R_{WT}), where a response of 1 (dashed line) indicates no change from wild-type *E. coli* cells. The colored bars in each panel indicate the molecules that are specifically recognized by the deleted chemoreceptor. The *cheY* mutant was included as a negative control (lower right panel), as this strain is motile but does not chemotax. Each value represents the mean of ten measurements ($n = 10$) performed on two separate assay devices. Error bars indicate the SEM.

Figure 4. Global chemotaxis response of wild-type *E. coli* MG1655 cells grown on different carbon sources. Cells were raised in M9 minimal media supplemented with 15 mM of the chemical indicated above each plot (every condition was also supplemented with 15 mM glycerol to support growth). Chemotactic responses were measured against the panel of molecules depicted in Figure 3A. Each value represents the mean of ten measurements ($n = 10$) performed on two separate assay devices. Error bars indicate the SEM.

Table 1

A list of half-maximal effective concentration (EC_{50}) and peak response values for each chemical that gave a measurable chemotaxis response. Values were derived from chemotactic response curves like those shown in Figure 2B (details are provided in the Supporting Information).

Chemical	EC_{50} [M]	Peak [M]	Chemical	EC_{50} [M]	Peak [M]
Amino acids					
alanine	2.1×10^{-3}	1.4×10^{-2}	Non-metabolizable amino acids		
arginine	–	$>1 \times 10^{-1}$	αMA	1.4×10^{-4}	3.3×10^{-3}
asparagine	7.0×10^{-4}	4.7×10^{-2}	AlBu	1.4×10^{-2}	4.1×10^{-2}
aspartate	1.8×10^{-5}	7.4×10^{-2}	Dipeptide		
cysteine	2.0×10^{-4}	7.5×10^{-3}	ProLeu	1.4×10^{-6}	2.8×10^{-5}
glutamate	8.5×10^{-4}	1.5×10^{-2}	Sugars		
glycine	4.1×10^{-3}	4.8×10^{-2}	galactose	1.6×10^{-6}	3.5×10^{-6}
methionine	–	$>1 \times 10^{-1}$	ribose	1.4×10^{-6}	3.0×10^{-6}
proline	–	$>1 \times 10^{-1}$	glucose	4.5×10^{-6}	1.5×10^{-5}
threonine	–	$>1 \times 10^{-1}$	Non-specific metabolite		
serine	2.4×10^{-4}	2.2×10^{-3}	succinate	1.6×10^{-2}	3.5×10^{-2}