

PERSPECTIVE

Effects of population density and chemical environment on the behavior of *Escherichia coli* in shallow temperature gradients

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Abstract

In shallow temperature gradients, changes in temperature that bacteria experience occur over long time scales. Therefore, slow processes such as adaptation, metabolism, chemical secretion and even gene expression become important. Since these are cellular processes, the cell density is an important parameter that affects the bacteria's response. We find that there are four density regimes with distinct behaviors. At *low cell density*, bacteria do not cause changes in their chemical environment; however, their response to the temperature gradient is strongly influenced by it. In the *intermediate cell-density* regime, the consumption of nutrients becomes significant and induces a gradient of nutrients opposing the temperature gradient due to higher consumption rate at the high temperature. This causes the bacteria to drift toward low temperature. In the *high cell-density* regime, interactions among bacteria due to secretion of an attractant lead to a strong local accumulation of bacteria. This together with the gradient of nutrients, resulted from the differential consumption rate, creates a fast propagating pulse of bacterial density. These observations are a result of classical nonlinear population dynamics. At *extremely high cell density*, a change in the physiological state of the bacteria is observed. The bacteria, at the individual level, become cold seeking. This appears initially as a result of a change in the methylation level of the two most abundant sensing receptors, Tsr and Tar. It is further enforced at an even higher cell density by a change in the expression level of these receptors.

1. Introduction

Temperature influences many important phenomena, such as chemical interactions, metabolism and dynamics. Therefore, almost all living organisms have developed the ability to sense and respond to temperature. Most multi-cellular organisms are

able to control their body temperature in order to withstand changes in their environment. Bacteria, on the other hand, have developed a mechanism to alter their swimming pattern in order to migrate toward a favored temperature.

Many studies aimed to understand the sensing and response mechanisms in bacteria [1–10]. They have identified the sensing receptors for temperature and the protein network controlling the response of the bacteria. We know that *Escherichia coli* (*E. coli*) bacteria have five receptors that sense

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temperature as well as chemicals [11–15]. These receptors are Tsr, Tar, Trg, Tap and Aer. The response of each receptor depends on the methylation state of the receptor, determined by the concentration of the different attractants and repellents in the surrounding medium [13–20].

Bacterial response to temperature is fast (less than a second). For sharp changes in temperature ($\sim 2^\circ\text{C}/100\text{ ms}$), bacteria will respond by changing their tumbling frequency as was measured in tethered bacteria [11]. On the other hand, in a shallow gradient ($\sim 3^\circ\text{C mm}^{-1}$), bacteria will have to travel long distances before they can sense any significant change in temperature. As a result, change in the swimming speed [21] or steady-state tumbling frequency [11, 21], methylation rate and even gene-expression as well as the chemical environment itself will influence the behavior of the bacteria in the temperature gradient. In addition, the chemical environment can be changed through consumption and secretion by the bacteria, and thus becomes a dynamical process that depends among other things on the rates of these activities, which depend on the bacterial cell-density. An important distinction is made here between nutrient-rich and nutrient-depleted environments because of the different effects that each type of environment has on the bacterial cellular activity.

We show here that the bacterial cell-density is an important factor that determines the behavior of bacteria in shallow temperature gradients. Using newly obtained data together with previous results, we distinguish between different density regimes. We characterize the behavior of the bacterial population and emphasize the important cellular activities that influence their behavior. A clear distinction is also made between the behavior of bacteria in a rich medium (M9CG medium, M9 minimal medium supplemented with 1 g l^{-1} casamino acids and 4 g l^{-1} glucose) and nutrient-free motility buffer (10 mM potassium phosphate, 0.1 mM EDTA, $1\ \mu\text{M}$ L-methionine, 10 mM sodium lactate, $\text{pH} = 7.0$). We conclude by building a comprehensive picture of the boundaries and conditions of bacterial life where the chemical environment and the slow physiological and metabolic processes become important. However, there remain many unsolved questions that we discuss and propose new experimental directions that we hope could answer them.

2. The various density regimes

2.1. Low cell-density

When the *wt RP437* bacteria are suspended in the M9CG medium and subjected to a linear temperature gradient, they accumulate around their favored temperature, $35\text{--}37^\circ\text{C}$ [21, 22]. Surprisingly, without nutrients, i.e. in motility buffer, they move to the highest temperature (figure 2). This result appears to contradict previous experiments conducted with tethered bacteria in the same medium, where measurements of the change in the bacterial tumbling frequency in response to a fast temperature impulse showed that 37°C is the optimal temperature [11]. The change in tumbling frequency is a measure that reflects the bacterial sensing network.

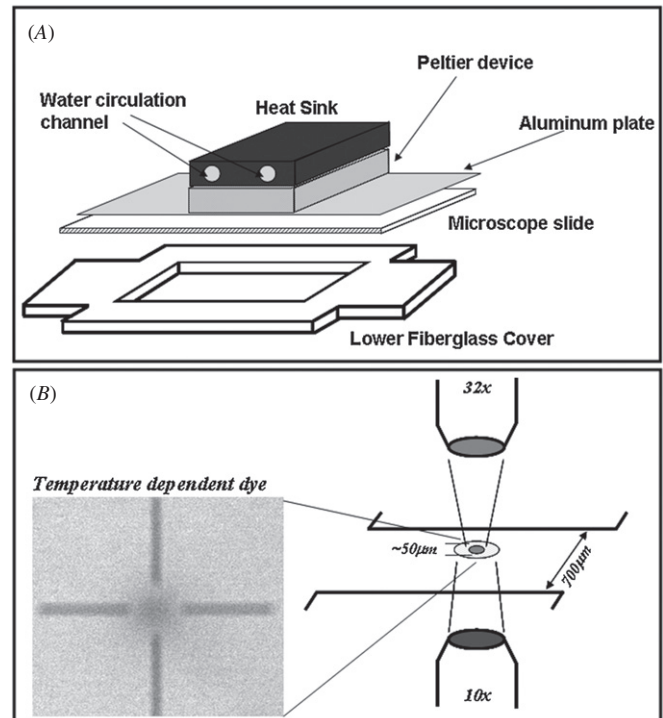


Figure 1. Experimental setups. (A) Homogenous temperature was applied using a Peltier device ($25\text{ mm} \times 25\text{ mm}$) attached to a thin aluminum plate covering the microscope slide. The Peltier device was attached on the other side to a heat sink consisting of a metal block held at 15°C by water circulation. All the thermal contacts were made using silicon-based heat sink compound (GC Electronics). (B) To create a sharp temperature gradient, an infrared laser ($\lambda = 1480\text{ nm}$) was focused into the sample through a $32\times$ objective. The background temperature was controlled via two aluminum blocks (dimensions: $W:25\text{ mm} \times L:25\text{ mm} \times H:10\text{ mm}$) attached to the end of the microscope, whose temperature was controlled by water circulation. This resulted in a Gaussian-shaped temperature gradient spanning $\sim 50\ \mu\text{m}$ radius as can be seen in the image depicting the fluorescence of a temperature-sensitive dye (BCECF, molecular probes; see [25]) while applying the laser to show the temperature profile. For applying a linear temperature gradient, a setup similar to that described in [22] was used. The bacteria in all experiments were viewed on an inverted microscope by fluorescence microscopy using a $10\times$ objective.

However, bacteria are swimming organisms and, as a result, the swimming speed and the viscosity of the surrounding medium, which are also physical phenomena affected by temperature, become important in shallow gradients. When the speed and the run time of bacteria in one dimension change with position, theoretical analysis showed that their flux is no longer described by Fick's law [23, 24]. In that case, the steady-state distribution of bacteria in a closed system will be inversely proportional to their speed [23, 24]. We have measured the bacterial speed at different temperatures (without gradient) using the setup described in figure 1(A), in both M9CG medium and in motility buffer. The results presented in figure 3(A) show that in M9CG, the speed of the bacteria (v) increases continuously with temperature, whereas in motility buffer it decreases for temperatures above $\sim 30^\circ\text{C}$. The speed measurement in motility buffer seems to contradict recently published results that show a continuous increase in

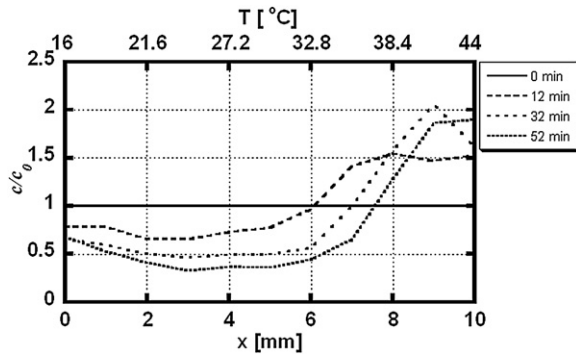


Figure 2. Bacterial concentration in a temperature gradient. Response of bacteria to a temperature gradient in motility buffer. The different lines depict the concentration profile of the bacteria at different times. The concentration of the bacteria at the different locations was estimated by recording fluorescence images, using a cooled digital CCD camera (SensiCam, The Cooke Corporation), and counting the cells per unit volume in each image. The increase or decrease in the number of cells in different areas was used to estimate the change in bacterial concentration [22, 26]. c_0 is the initial concentration of bacteria in the channel. The temperature gradient in the channels was measured using the temperature-sensitive fluorescent dye (BCECF, molecular probes; see [25]).

the bacterial speed with temperature even without nutrients added [30]. This could be due to differences in measurement methods used in both cases. Note that in our measurement, we avoided tracking bacteria near the surfaces to avoid surface effects, and we included all intervals of the bacterial motion including the intervals where the bacteria seemed just to tumble. Our reasoning being that during tumbling, the bacteria can still drift due to diffusion, and we are interested in the contribution of all components of the bacterial motion and not only those controlled by the bacterial flagella.

The distributions we observe in figure 2 (and previous works [22]) do not reflect the spatial dependence of v (figure 3(A)). If we assume that the changes in speed and run time occur over longer time scales (adaptation time scales), then the bacterial flux can be described by Fick's law with position-dependent effective diffusion coefficient (D_{eff}). D_{eff} accounts for all parameters that influence motility [27, 31]. In addition, objects with a spatially dependent D_{eff} will spend more time at regions with lower D_{eff} , which will cause accumulations where D_{eff} is smaller. This, however, will not occur in pure diffusive closed systems with a flat energy landscape since a pure diffusion is described by the general diffusion equation $\partial n/\partial t = \nabla(D_{\text{eff}}(T(x))\nabla n)$, whose steady-state solution will always be $n(x) = \text{constant}$. On the other hand, bacteria do sense temperature and follow temperature gradients. In a shallow temperature gradient, even though the bacterial sensing is small, it can cause the bacteria to migrate and the spatial dependence of their diffusivity can cause them to spend more time where it is minimum leading to the observed accumulation. Measurements of D_{eff} presented in figure 3(B) show that in the M9CG medium, D_{eff} exhibits a minimum at $\sim 37^\circ\text{C}$, whereas in motility buffer, it continues to decrease for temperatures as high as 42°C . These results could explain the observed spatial distribution of bacteria in the temperature gradient.

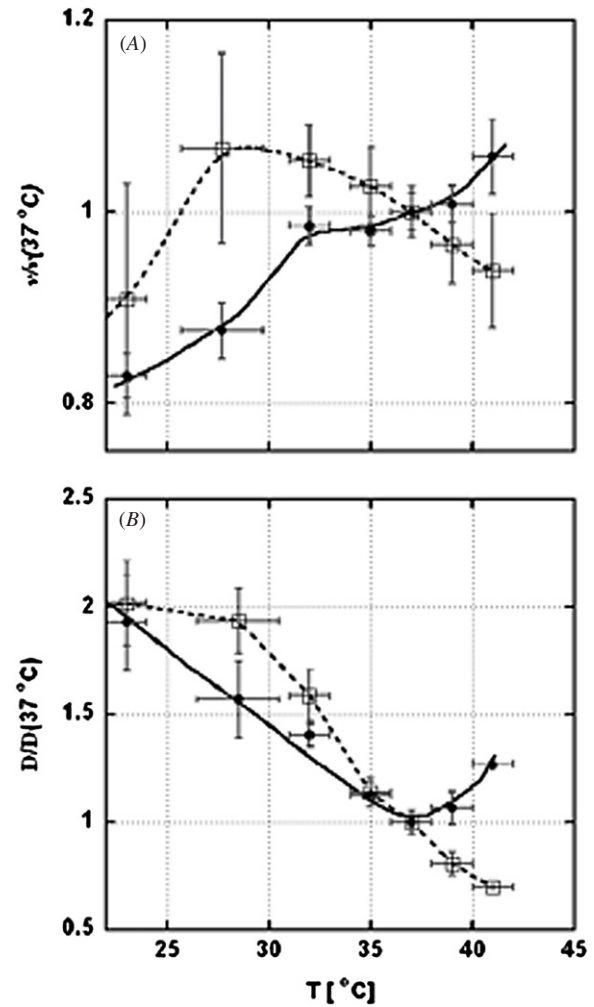


Figure 3. Velocity and effective diffusion coefficient versus temperature. (A) The velocity as a function of the temperature in M9CG medium (full circles) and in motility buffer (empty squares). To calculate the average speed of bacteria as a function of temperature, we used trajectories acquired from the analyses of movies of swimming bacteria using ImageJ software (NIH) [27, 28]. The distances traveled by each bacterium between two consecutive frames were calculated, averaged and divided by the time interval between the frames (100 ms). When the cells did not move between two consecutive frames, the measurement was not included. (B) D_{eff} of wt bacteria, RP437, as a function of temperature in the M9CG and in motility buffer (circles and squares like in A). D_{eff} was calculated by fitting the mean-squared displacement of the bacteria as a function of time to $\langle \Delta r^2(t) \rangle = 4D_{\text{eff}} t(1 - \exp(-t/\tau))$ [29], using Mathematica (Wolfram). The mean-squared displacements were calculated using the same trajectories acquired using ImageJ. All recordings for these measurements were acquired ~ 2 minutes after the temperature was stabilized using a cooled digital CCD camera (SensiCam, The Cooke Corporation).

To emphasize the role of the sensors in the observed behavior, we used a mutant strain (UU2616) with all sensors deleted. We find that D_{eff} decreases continuously with increasing temperature and no minimum is observed at 37°C (figure 4(A)). Furthermore, in a linear temperature gradient (figure 4(B)), the sensorless bacteria move toward the higher temperature and do not accumulate around 37°C , just as would be expected based on the above explanation. On the other hand, adding the non-metabolizable ligand

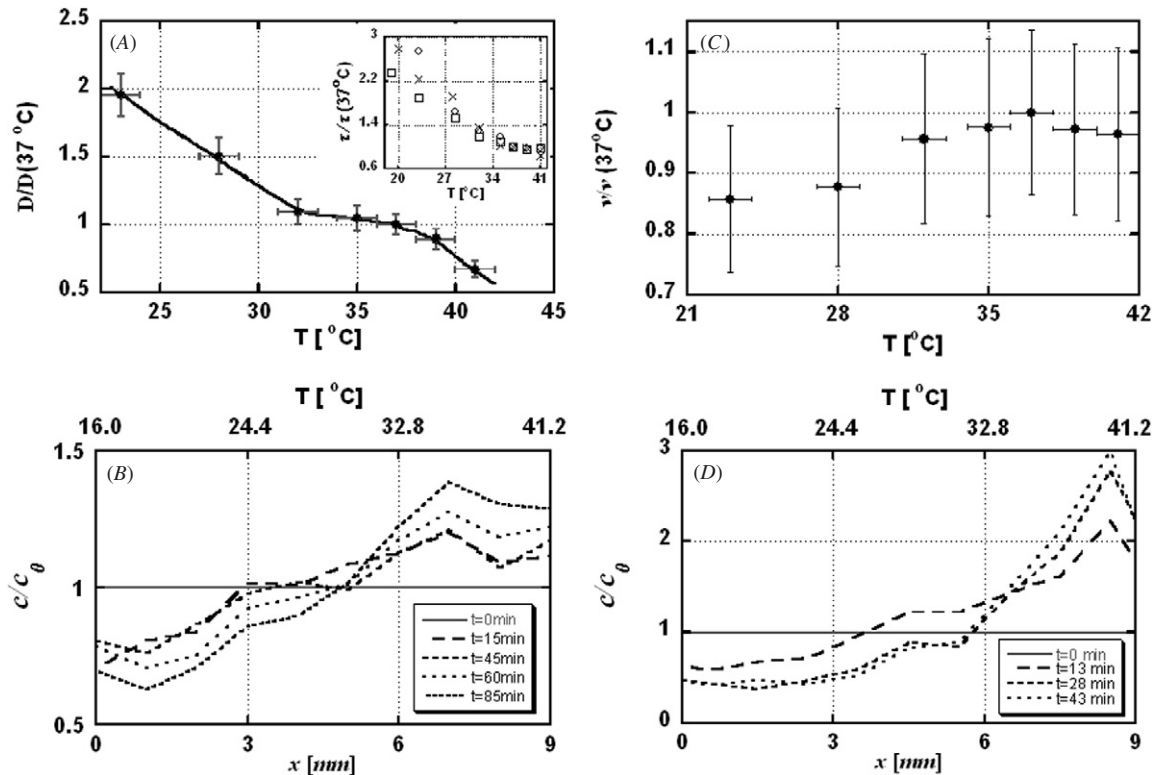


Figure 4. The response of a UU2612 to a temperature gradient. (A) Effective diffusion coefficient versus temperature of the mutant strain UU2612 (with all the receptors deleted) in M9CG. The inset exhibits the correlation time (τ) as a function of temperature for the UU2612 bacteria in the M9CG medium (\times), RP437 in motility buffer (empty squares) and RP437 in the M9CG medium (empty circles). (B) The change in the concentration of a mutant strain UU2612 (with all the receptors deleted) along a linear temperature gradient. The bacteria were suspended in the M9CG medium at an optical density of 0.3. c_0 is the bacterial concentration at the beginning before applying the gradient. (C) The change in the speed of the mutant strain UU2612 in the M9CG medium as a function of temperature relative to its speed at 37°C . (D) The same as (B) but for the wild-type bacteria RP437 suspended in motility buffer with $500\ \mu\text{M}$ MeAsp.

α -methyl-aspartate (MeAsp) to the bacterial culture in motility buffer at a concentration of $500\ \mu\text{M}$ shifts the accumulation of the bacteria to a slightly lower temperature (figure 4(D)) than observed for the wild-type strain in motility buffer (figure 2). MeAsp is known to increase the methylation state of the Tar receptor and therefore changes its temperature preference from warm to cold temperature [17–20]. This emphasizes that the bacterial sensing (and not only the effective diffusivity) is important to achieve accumulation at the favored temperature in a shallow temperature gradient.

Finally, since $D_{\text{eff}} \approx v^2 \cdot \tau$, where τ is the mean free path time (defined in the equation in figure 3 caption), the difference between D_{eff} of RP437 in M9CG on one hand and D_{eff} of RP437 in motility buffer and D_{eff} of UU2612 in M9CG on the other hand could be due to the change in v or τ . We find though that the change in τ as a function of temperature is the same for all strains and conditions (figure 4(A) inset). This implies that the observed difference in D_{eff} is due to the change in the behavior of the speed v as a function of temperature for the different strains and in the different media. Figure 4(C) shows that the speed of the mutant strain UU2612 in the M9CG medium decreases for high temperatures in a similar manner to the wild-type strain in motility buffer. It is important to note here that the effect of deleting the sensing receptors on the bacterial swimming speed (as can be seen in figure 4(C))

is not yet understood. However, these results indicate that the receptors as well as nutrients play a role in controlling the bacterial speed. Studies to test this theory are currently underway and preliminary results support this hypothesis.

2.2. Intermediate cell-density

An important effect of temperature is in changing the metabolism of bacteria. If the bacterial concentration is high enough, this change in metabolism creates a gradient of nutrients that will affect the bacterial behavior in a temperature gradient. Previous studies [22] have shown that when subjected to a shallow temperature gradient in a nutrient-rich medium, bacteria initially accumulate around their favored temperature, but later drift toward the lower temperature regime (figure 5(A)). The drift is attributed to the faster depletion of nutrients at a higher temperature. In order to further explore this effect and confirm that bacterial metabolism in a temperature gradient will create a nutrient gradient opposite to the temperature gradient, we have performed new measurements of the bacterial consumption rate as a function of temperature. It is difficult to experimentally measure the change in concentration of nutrients. As we have developed a sensitive oxygen sensor [35], oxygen is used here as the ‘nutrient’, and we measure its level in time and its dynamical evolution in order to estimate the effect of temperature on the bacterial consumption rate. In

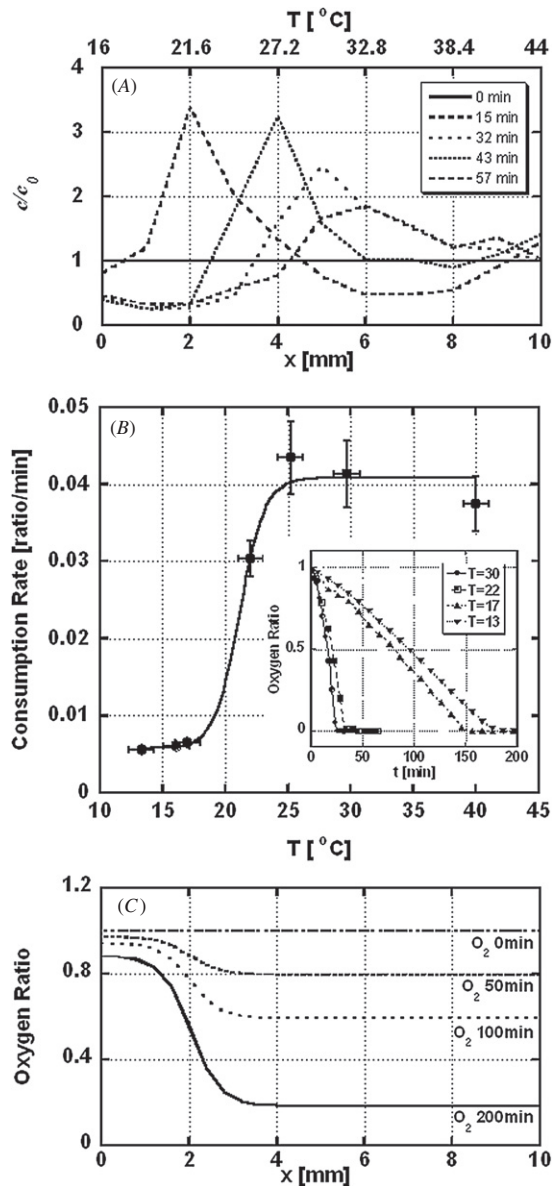


Figure 5. The effect of temperature on oxygen consumption. (A) Examples of the response of bacteria to a temperature gradient in the M9CG medium. The different lines depict the concentration profile of the bacteria at different times. (B) The consumption rate of oxygen by *E. coli* in a motility buffer as a function of temperature in units of ratio of initial oxygen concentration. The solid line represents the best fit to the data given by $0.00577 + \frac{0.0351}{1 + e^{-(T-21.12)}}$, where T is the temperature. The consumption rates were estimated from the measurements of the change in the oxygen concentration in a closed sample where oxygen cannot diffuse in or out. The oxygen concentration in the solution was measured using a newly developed biocompatible and water-soluble dye (Ru-micelle), based on the spontaneous formation of phospholipid micelles around the hydrophobic Ru(dpp) molecules [27]. Example measurements are depicted in the inset. (C) Calculation based on the data from (B) of the expected shape of the oxygen gradient at different time points when the bacteria are subject to a temperature gradient. The temperature gradient, used in this calculation, is $T = 15 + 3 \cdot x$, where T is the temperature in degree celsius, and x is the location in millimeter.

another study, a similar drift toward the lower temperature was attributed to an oxygen gradient [21].

Our measurements, presented in figure 5(B), show that as the temperature increases, the rate of oxygen consumption by bacteria increases. The depletion rate of oxygen was estimated by measuring the change in the oxygen concentration in an isolated bacterial solution at different temperatures as shown in the inset of figure 5(B). From these measurements, we conclude that in a temperature gradient, the change in the depletion rate of oxygen as a function of temperature would result in an oxygen gradient opposite to the temperature gradient as depicted in figure 5(C). The time scale, over which a significant change in the oxygen concentration appears, depends on the bacteria's concentration. In the case presented in figure 5(C), where the bacteria's concentration is $\sim 5 \times 10^6$ cells cm^{-3} , a considerable change in the oxygen level at different locations along the temperature gradient appears only after a few tens of minutes, whereas the accumulation of bacteria at their favored temperature is faster (on the order of minutes). Therefore, the effect of consumption becomes important only later in time. At low cell-density, $\sim 0.5 \times 10^6$ cells cm^{-3} , this effect is not observed as it takes ~ 500 min to establish a considerable gradient.

2.3. High cell-density, cell-cell interaction

Bacteria change the chemical content of their surroundings also by secreting chemicals [32]. In a previous study, we have shown that when the concentration of the bacteria is increased beyond 10^8 cells cm^{-3} , a drastic change in the behavior is observed [22]. Our results showed that the concentration peak, formed around the bacterial favored temperature in the early stages, develops rapidly into a sharp and fast propagating pulse. The propagation speed was $\sim 3.5 \mu\text{m s}^{-1}$, which is close to the maximum velocity of a single bacterium chemotaxing up a gradient of nutrients. This new mode is due to bacterial secretion of an attractant amino acid, glycine. This was confirmed by the observation that the sharp accumulation of bacteria was diminished when glycine was added to the medium at a high concentration preventing the bacteria from sensing small changes in its concentration [22].

The concentration of secreted glycine in the medium should follow the concentration of the bacteria. However, if the secretion rate of glycine is slow relative to its diffusion, then the secretion will not create a glycine gradient. Therefore, this effect is important at very high cell-density, where the secretion rate is large enough to establish a gradient of glycine. As a result, bacteria sense the glycine gradient and are attracted by it, which creates a positive feedback loop that amplifies the initial accumulation of bacteria. This amplification leads to a sharper and denser accumulation and therefore a faster consumption of nutrients in the region of the accumulation. The fast consumption in turn creates a sharper nutrient gradient and hence a faster traveling peak, moving toward higher nutrient concentration at a lower temperature. Similar behavior was observed previously as a result of stochastic fluctuations in bacterial concentration due to boundary conditions, which led to a sharp accumulation as

a result of attractant secretion. This accumulation propagated in the sample in the form of a pulse due to subsequent faster depletion of nutrients at the location of the bacterial accumulation and the creation of a gradient of nutrients [32]. Another system where similar behavior was observed is in the swarming of bacteria [33, 34]. There too, the expansion of the swarm ring was attributed to the consumption of nutrients at the center of the ring creating a gradient of nutrients that directs the expansion observed. This collective mode is a classical behavior observed in many cases of population dynamics as a result of strong interactions between individuals.

2.4. Extremely high cell-density, methylation effect

To this end, we have described the dynamics of the bacterial population as a function of its concentration where changes in the environment or changes in the population density do not alter the physiological state of the bacteria. At extremely high concentrations of bacteria, this is not true anymore. Previously, we showed that when bacteria are grown in the M9CG medium, the accumulation of secreted glycine in the medium increases the methylation level of the Tsr receptors and causes the bacteria to switch their thermal preference to lower temperature [26]. This switch occurs after the bacteria grow beyond a critical concentration.

In a series of new experiments, we explored the relationship between this critical concentration (at which the bacteria became cold seeking) and the concentration of nutrients in the growth medium. We measured the response of the bacteria to a fixed temperature gradient between 30 and 18 °C. The bacteria were taken at different time points during their growth, resuspended in fresh M9CG medium, and the rate of their accumulation at (or depletion from) 30 °C was measured (for more details, see [26]). As observed previously [26], at the early stages of their growth (or low concentration), bacteria swim toward the high temperature, whereas later at a very high concentration, they swim toward the low temperature. The concentration, at which bacteria started to swim away from the 30 °C region, increased in a nonlinear way with the initial concentration of nutrients in the growth medium (figure 6(A)).

Note, however, that the growth rate of the bacteria in all three cases is exactly the same (see figure 6(A) inset). Therefore, one can assume that the secretion rate of glycine is the same for all initial concentrations of nutrients. If the concentration of glycine was the only factor responsible for the switch, then there should be no change in the switching point. This indicates that other factors are involved, and that the change in the physiological state of the bacteria is not fully understood and further investigation is required. The use of a minimal medium, instead of the complex one used here, where the effect of single nutrient at a time can be studied should help reveal the important nutrients controlling this switch and eventually help model this behavior.

2.5. Extremely high cell-density, expression level of the sensing receptors

In addition to all the above-mentioned effects, there are other changes that occur in the bacteria, at much longer

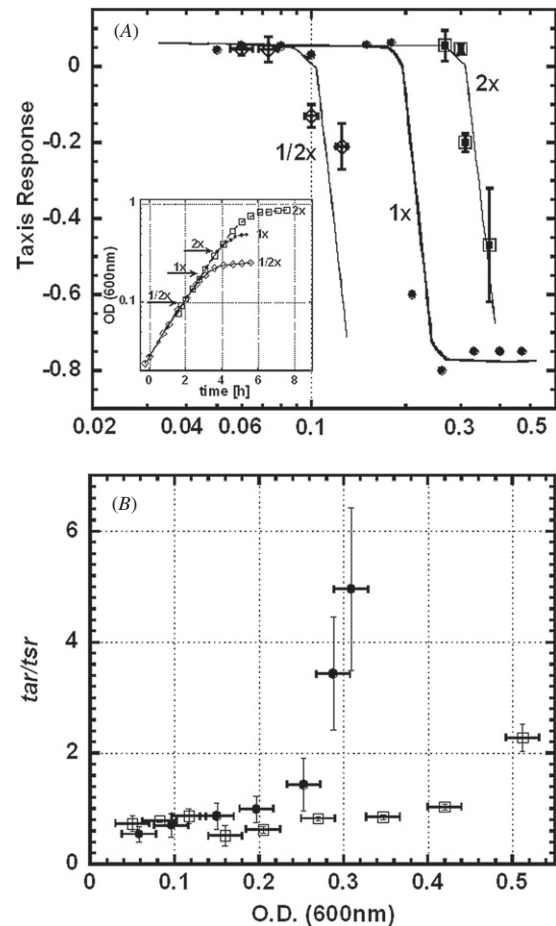


Figure 6. The effect of nutrients on the receptor transcription ratio and the switching point. To quantify the response of the bacteria to a temperature gradient, the background temperature was held at ~ 18 °C and the laser was applied (as described in figure 1) to heat the center to ~ 30 °C. Movies of the fluorescence were recorded. The increase or decrease in the fluorescence signal at the heated region relative to the background was measured. The change in the fluorescence signal was fitted to an exponential function, which was used as the quantitative measure of the response. An accumulation (or attraction to heat) results in a positive exponent, whereas depletion (or escape to cold) leads to a negative one. (A) The exponents representing the taxis response of the wt RP437 bacteria to laser heating as a function of the optical density of the bacterial culture from which the sample was taken are presented. The bacteria were grown with different initial concentrations of casamino acids and glucose (diamonds $1/2\times$, circles $1\times$ and squares $2\times$, where \times is 1 g l^{-1} casamino acids and 4 g l^{-1} glucose). The lines are hand drawn as guides to the reader. The inset depicts the growth curves of the same bacteria under the different conditions mentioned above. The arrows point to the optical density at which the bacteria switch from warm to cold seeking bacteria for each of the growth conditions. The results for $1\times$ are taken from [26] for comparison purposes. (B) The Tar/Tsr ratio as a function of the optical density. The squares are for the bacteria grown in the M9CG medium and the circles are for the bacteria grown in the same medium with half of the initial concentrations of casamino acids and glucose. Measurements were carried out using a real-time PCR technique as described in a previous publication [26].

time scales (generation time scale), due to alterations in the expression level of the sensing receptors [26]. This, as we have demonstrated in the past, changes the response of the bacteria to temperature gradients in general.

Measurements of the average expression ratio of the two most abundant receptors in the bacteria, Tar/Tsr, showed that there is a drastic change in this ratio as a function of cell density. This change can alter the response of the bacteria not only to temperature gradients but also to chemical gradients [35]. Therefore, it is important to understand what controls this change in the average ratio of the sensing receptors Tar/Tsr. To begin answering this question, we have again studied the effect of nutrients. We measured the ratio of Tar/Tsr expression levels in two growth media that differ in the nutrients' concentration. Our recent results presented in figure 6(B) show that decreasing the initial concentration of nutrients in the growth medium by half, namely casamino acids and glucose, caused the change in the Tar/Tsr ratio to appear at lower cell-density. The mechanism underlying this change, which can be seen as a long-term memory of the bacteria to their chemical surrounding, is still unclear and subject to ongoing studies. Again, a systematic study of the effect of each nutrient on the Tar/Tsr ratio should be undertaken. This also raises the question of whether the switch in the response to a temperature gradient reported in the previous section occurs at a particular ratio Tar/Tsr. Answering this question will help reveal whether Tar and Tsr have similar thermal sensitivity and will allow us to estimate the relative strength of their sensitivities.

3. Summary and discussion

The results presented here together with previous studies [22, 26] show that the bacterial cell-density plays an important role in determining the response of the population to a shallow temperature gradient. Temperature affects the bacterial cellular activity. Changes in cellular activity can lead to changes in the chemical environment, which eventually interfere with the bacterial thermo-sensing and can cause the bacteria to swim toward different temperature regions. Increasing the bacterial concentration increases the rate of change in the chemical environment and the level of interactions among individuals. This in turn leads to a more complex behavior up to changes in gene expression. The processes responsible for this increased complexity occur on long time scales and therefore are important in shallow temperature gradients only.

The distinction made here between the different cell-density regimes is also interesting because it clearly separates biological from dynamical effects. Many of the changes in the dynamical behavior of the bacteria observed as a result of increasing the cell density are common to many-body systems. Specifically, the behavior observed in the first three examples is dynamical. The bacteria initially act as individuals and inter-body interactions are negligible. As their concentration increases long-range interactions become significant. This type of long-range interaction among bacteria is observed in the population drift toward lower temperature as a result of temperature-dependent nutrient depletion. Further increase in cell density gives rise to the short-range interactions affecting neighboring bacteria and causing local aggregates to amplify.

The sharp accumulation of bacteria is a result of this short-range interaction caused by the secretion of an attractant amino acid. On the other hand, the changes in the behavior of the bacteria described in the last cases are biological and do not resemble any physical phenomena. These changes are due to modifications in the physiological state of the bacteria; first by methylation of the receptors, and then as a result of changes in gene expression. The extent of the change in the physiological state of the bacteria and its effects on the behavior of the bacteria under different conditions are still under investigation.

Finally, this study raises many questions. Most importantly, what exactly causes the change in the physiological state of the bacteria, and how does this mechanism allow the bacteria to adapt to the stress in the environment? In addition, how do bacteria integrate thermal and chemical signals to determine the output response? How do chemicals influence the response of the bacteria to temperature gradients at the single-cell level? What is the mechanism controlling the expression level of the sensing receptors and how is it influenced by the temperature and the chemical content of the medium? These questions beg for new investigations.

Acknowledgments

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