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The Motility of Bacteria in an Anisotropic Liquid Environment

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Although the influence on bacterial motility of many genetic and biochemical factors has been extensively studied, there have been limited studies of the impact of physical parameters. Indeed, despite the fact that natural environments are often asymmetric (such as stretched supramolecular structures), the majority of behavioral experiments with bacteria have been done in isotropic liquid solutions. In the present work, we show that the behavior of living microorganisms is dramatically different in media that are asymmetric. The example of Escherichia coli *bacteria swimming in a bulk uniaxial liquid environment is used to demonstrate this phenomenon. The results of our study provide insight into the behavior of bacteria in conditions encountered in real environments and open new avenues for the control of their movements.*

Keywords *E. coli* bacteria; liquid crystal; microorganism; motility; orientation; single cell tracking; symmetry; viscosity

Introduction

There are 10 times more bacteria in a human body than the number of cells [1]. The microbial cells (and viruses) that live in and on the human body, collectively referred to as our microbiota, play a major role in our health and have been linked to immune diseases [2] such as allergies and asthma [3], obesity [4], and many other conditions [5]. The mechanisms of bacterial colonization of our body (host) were studied mainly from a biochemical point of view [6–8]. The only physical parameters that have been studied so far in relation to bacterial motility (speed and path length) are the viscosity of the host [9–11] and the presence of surfaces or microchannels [12–14]. All other studies of the dynamic behavior of microorganisms, such as *Escherichia coli* (*E. coli*) bacteria, have been done in environments that are *macroscopically isotropic* (including cases of locally heterogeneous media, like agar). While those studies have led to spectacular progress in understanding many fundamental aspects of behavior of microorganisms, these studies do not fully describe how microorganisms behave in real conditions, in the living tissue environment that often has a locally broken symmetry. In fact, many intrinsic self-assembly properties

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are driving the local angular ordering (orientation) of biological macromolecules, which may break down the local symmetry. For example, many angularly correlated molecular or supramolecular systems may be found in the human body, ranging from the DNA and cell membrane up to the tendon. Particularly well-known examples (not necessarily from a bacterial activity point of view) of such oriented systems are based on the collagen and the chitin, which support the long-range tridimensional spatial and orientational organization [15]. This type of organization often makes the local environment for living microorganisms strongly anisotropic exhibiting different mechanical, optical, and diffusive properties along different directions.

To simulate such anisotropic host conditions for bacteria, we have used a biocompatible liquid crystal (LC) host. The specificity of this material comes from the orientational organization of its molecules in the water solution that creates strong angular correlation of their cylindrical aggregates leading to a local anisotropy axis in the liquid phase of the material. The commonly used term to describe this axis is the *director* (usually showing the local orientation of the anisotropy axis of the LC [16]). Another unique feature of LC materials is the orientational flexibility of the director that may be deformed (reoriented) easily, e.g. by locally applying a mechanical stress or electric or magnetic torques. Also, the molecular diffusion is anisotropic in these materials. The angular flexibility of the director has very interesting electro-optical properties, which have enabled such important applications as the LC displays (LCDs). This flexibility of the director as well as its sensitivity to various stimuli has also recently triggered the interest in using LC materials for biomedical sensing applications [17–20]. It is however important to note that the LC character of the host was used (in above-mentioned works) only for the detection of the presence of microorganisms by the molecular misalignment or by distortion of the director of the LC.

In the present work, we show that LC materials may also be used as hosts to better understand and control the *behavior* of biological microorganisms (the particular example of *E. coli* bacteria is used). Moreover, we show that key motility parameters are dramatically changed in such hosts. We think that the anisotropic diffusion processes and elastic (orientational) distortions of the host play an important role in these changes. We will however describe here only the behavioral changes of the bacteria and leave the detailed theoretical analyses (such as energetic balance, etc.) for a later publication.

Materials and Methods

Escherichia coli strain HCB33 (= RP437, wild-type for motility and chemotaxis) was streaked on 1.5% agar (Difco) containing T-broth (1% Tryptone [Difco], 0.5% NaCl) and grown at 37°C overnight. A single-colony isolate was used to inoculate 10 mL of T-broth in a 125-mL flask, and the culture was grown to saturation on a rotary shaker (200 rpm) at 34°C. An aliquot was diluted 1:100 into another 10 mL of T-broth and grown 4.5 h to midexponential phase (OD 0.4–0.6, or about 3×10^8 cells/mL). Finally, the bodies of the bacteria were made fluorescent by adding to the solution 0.01 mM of SYTO 9-green (from the Invitrogen Live®Dead kit), a fluorescent dye (peak excitation at 480 nm and emission at 510–540 nm) that freely diffuses through cell membranes.

As the LC host, we have chosen the water solution of a biocompatible compound, called DSCG (Cromolyn sodium salt CAS Number 15826-37-6), which was purchased from Sigma Aldrich. This material is a lyotropic chromonic LC (LCLC) and has already been studied in several works, see e.g. [17,19,22]. We have prepared distilled water solutions of

DSCG with different concentrations. Those solutions were then filtered (with pore diameters $\emptyset \approx 0.2\ \mu$m) to remove impurities and large aggregates. Fluorescent *E. coli* bacteria were finally added into the DSCG solution at small concentration so that the final concentration of DSCG in water was between 6% and 17% by weight.

The sandwich-like containers of the final solution were fabricated by using two coverslips of 170 μm thickness. To clean them, the coverslips were first sonicated in Micro 90, followed by water, acetone, and isopropanol in an ultrasonic bath for 5 min each, and finally dried in an oven. The glass coverslips were spin-coated with 1% v/v polyimide PI-150, dried at 80°C for 15 min, and then baked at 280°C for 1 h. The polyimide surfaces were mechanically rubbed to form a uniform alignment layer for the LCLC. Two identical coverslip substrates were put parallel (with rubbed polyimide layers facing each other and with their rubbing directions aligned parallel) and were fixed with UV curable glues (Norland UV sealant) dispensed along the edges of the substrates. The cell gap was controlled with 50 μm spacers, dispersed in that glue. The final LCLC solution, containing fluorescent *E. coli* bacteria, was allowed to flow by capillarity (parallel to the rubbing direction) into this optical container.

The bacteria were observed using an inverted microscope (IX71 Olympus) equipped for fluorescence microscopy with a 100X, 1.3 NA objective. Short videos, showing the movements of swimming bacteria, were recorded at 30 frames per second with a CCD camera (DMK 31BU03, Imaging Source). These videos were then analyzed with an *ImageJ* tracking plugin (a modified version of the ParticleTracker described in Ref. [21]) to obtain the position and orientation of each cell as a function of time (see Fig. 1). For each individual trajectory, the average speed was computed. Considering the standard deviation as the error on each of these values, a weighted average of all those mean speeds for tracks at the same concentration was then obtained (see Fig. 2). A similar procedure was followed to process the standard deviation of the cell body orientation for each track to generate Fig. 3. The cells were also viewed (by means of Zeiss microscope) between two crossed polarizers to image the LC texture and director orientation in the LCLC.

Results and Discussions

We started our investigation by observing the movement of bacteria in the two distinct phases of the host used: in the isotropic and liquid crystalline phases, at DSCG concentrations, below and above 12%, respectively [22]. Video analysis was used to track the position of the center of mass of individual bacteria as a function of time. Figure 1 shows a superposition of many of the resulting trajectories in the two situations and it illustrates a dramatic change in behavior across the phase transition of the host.

In the isotropic phase (Fig. 1a), we tracked cells at DSCG concentration of 6%, 8%, and 9%. We also observed bacteria in motility buffer (10 mM potassium phosphate, pH 7.0, 0,1 mM EDTA, 10 mM lactic acid, 0% of DSCG) to confirm that the presence of these low concentrations of DSCG had no effect on the motility. In all these cases, the bacteria's movements describe a *random walk* as described in the literature [23] (r^2 growing linearly with time). In contrast, the behavior of bacteria is dramatically changed (Fig. 1b) in the liquid crystalline (anisotropic) phase of the host, observed at $\geq$12% of DSCG. Two experimental challenges that were taken into account are worth mentioning here. First, the long scale orientation of the director of this material is more complicated compared to traditional *thermotropic* LCs. However, we have succeeded to obtain relatively well-aligned zones (with low director gradients) that were large enough (hundreds of micrometers) to observe the movement of bacteria in uniformly aligned areas. Second, the precise control

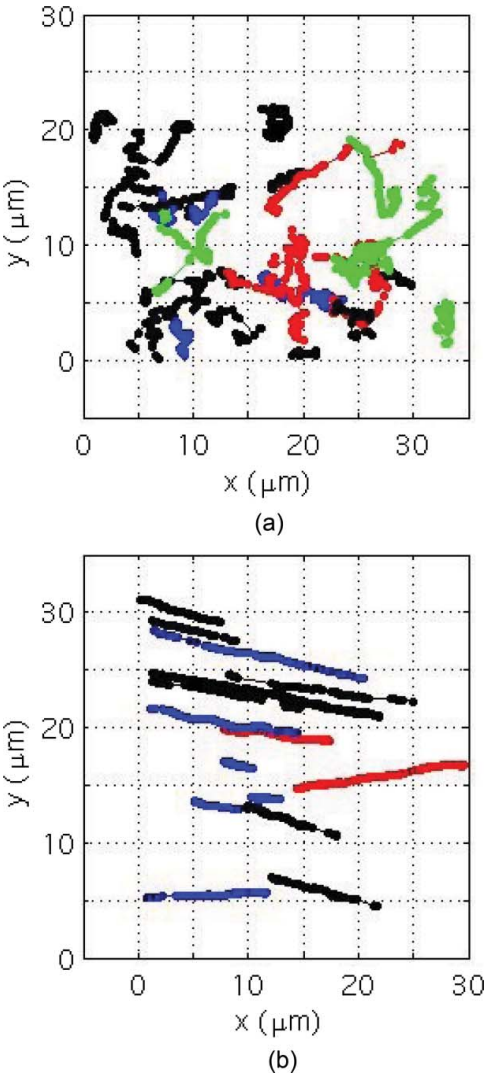

Figure 1. Superimposed paths of swimming bacteria (extracted by tracking the center of mass of fluorescent bacteria in recorded videos of 10 to 30 seconds at 30 frames per second) in (a) isotropic medium (0%, 6%, 8%, and 9% concentration of DSCG for the black, blue, red and green curves, respectively) and (b) anisotropic medium (14, 16, and 17% concentration of DSCG for the red, blue, and black curves).

of the DSCG concentration is rather difficult. This is the reason why we performed our experiments with mixtures having DSCG concentrations far enough from the critical values (12%) to be sure that the host is completely in the isotropic or anisotropic phase.

Our observations show (Fig. 1b) that in the anisotropic phase of the host bacteria are strongly constrained to move back and forth along "one-dimensional tracks" and their orientation is defined by the local director of the LC. Additional observations using a polarization microscope confirm this statement. The fact that the traces are not perfectly parallel in Fig. 1b is related to the slight gradient of the director. To avoid the possible

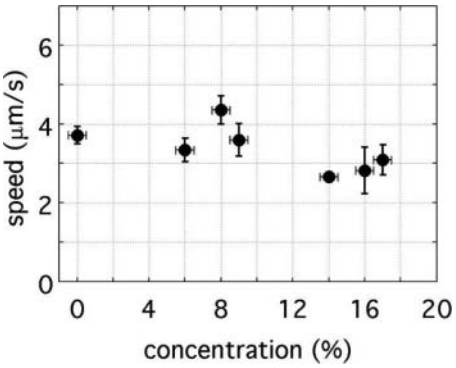

Figure 2. Average swimming speed of bacteria as a function of the concentration of DSCG. No drastic change is observed across the LC phase transition (around 12%). Error bars represent the standard error of the mean at each concentration.

effect of surfaces, only the bacteria swimming in the middle of the cell were observed. By swimming up or down, bacteria could therefore move out of focus, which is why some traces are shorter.

Interestingly, in contrast to the dramatic change in the directionality of the tracks, we do not observe a drastic change in the bacteria's swimming speed across the phase transition (at 12% DSCG), as shown in Fig. 2. The slight decrease in speed, if confirmed with more statistics, could be explained by an increase in the viscosity of the host medium. In fact, the effect of viscosity on bacteria motility has been studied for isotropic host media (see for example refs. [9,10]). In our case, we see that changes in DSCG concentration, large enough to transform our host from its isotropic state to its anisotropic state, do not affect dramatically the average speed of bacteria. The relationship between the viscosity and the concentration of DSCG is not obvious because of the structural specificities of this host (colloidal liquid composed of oriented large molecular aggregates). Once we have understood this relationship, we will be in a better position to analyze the dependence of the swimming speed with concentration of DSCG.

Our tracking algorithm also provided the cell body orientation angle for each tracked bacteria at each frame (obtained from the second order intensity moments of the image). We used that information to quantify the qualitative difference observed between the track in isotropic and anisotropic media shown in Fig. 1. We simply computed the standard deviation of all the angle measurements for a given track and averaged all these standard deviations for all the tracks recorded at the same concentration. The resulting values are displayed in Fig. 3. That plot unambiguously shows the change in bacterial behavior at the phase transition that the host undergoes at concentration of about 12%. In the isotropic phase (below 12% of DSCG), the orientation of the cell bodies quickly fluctuates over the entire range of possible angles (between 0° and 180°) whereas the angle of their orientation is much more stable (standard deviation of $\approx 18^\circ \pm 5^\circ$) in the LC phase (above 12%). In fact, above this critical concentration, the bacteria essentially swim along the *director* axis.

We think that, while several physical parameters undergo important changes in the LC phase of the host (such as the molecular aggregation size, anisotropy of diffusion, the increase of the shear viscosity, etc. [22]), the above-mentioned behavioral changes are related, to some limited extent, to the anisotropic diffusion, but to a greater extent, to the reduction of the elastic (director's deformation) energy required for these bacteria to move

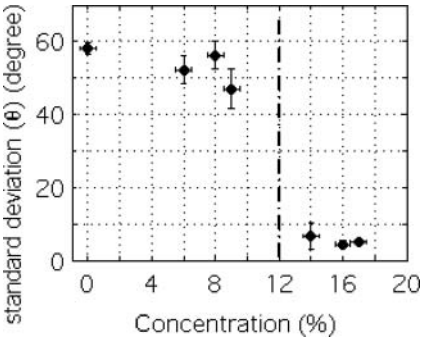

Figure 3. Standard deviation of the orientation of the bacteria's body for various concentrations of DSCG, below and above the LC phase transition (happening at approximately 12%, shown by the vertical dashed line). At each concentration, 5–10 bacteria were tracked for an average of 120 frames and the standard deviation of their body's angle vs. time were averaged to produce this plot. Multiple similar measurements have been done. The error bars on each point represent the standard error on the mean.

in the host. In fact, given the elongated shape of the bacteria's body (approximately 1 μm of diameter and 4 μm of length) and their movement being performed by propulsion (because of the rotation of their rigid helicoidal flagella), the distortion of the director should require higher energy if the bacteria were to try to move in the perpendicular direction. However, at this stage, it is difficult to observe directly and quantify this distortion since the size of the bacteria is very small and the nature of anchoring of the LC director on the "surface" of the bacteria is yet unknown to us. The study of this distortion and of the corresponding energetic balance is under way (see also [18]). Another interesting question under study is how the bacteria reverse the direction of their movements in such anisotropic media (maybe similar to the case of densely packed swarming bacteria, described in Ref. [24]).

In summary, we studied the behavior of *E. coli* by measuring the traces of bacteria, their speed, and their cell body orientation as a function of time in a biocompatible LC host for various concentrations of DSCG. We found that the trajectories and orientation of bacteria abruptly change with the orientational phase transition of the host. Thus, we have shown that above the critical concentration of the DSCG (bringing the host to its liquid crystalline *anisotropy* phase), the flagellated bacteria *E. coli* are constrained to mainly move back and forth along linear paths well aligned parallel to the local anisotropy axis of the host (both the bacteria tracks and their body's alignment).

To our best knowledge, this is the first report of such dramatic change in the microorganism's behavior caused by fully controllable properties of the host. These exciting results open the door for studying the behavior of other biological microorganisms in such environments that mimic much better the biological tissue, in the proximity of which, not only we can have viscosity change, but also a symmetry breakdown in terms of alignment of tissue constituents. Our approach could also provide a new avenue to control the movements of swimming cells by changing, for example, the orientation of the director with electric or magnetic fields. Indeed, the knowledge accumulated over the last 40 years of LCD research could be quickly leveraged for understanding and controlling the behavior of microorganisms in LC environments. We are convinced that the behavior of self-propelled microorganisms in anisotropic environments is a very rich and exciting research area that we are only beginning to explore.

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