Collective motion in an active suspension of *Escherichia coli* bacteria

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Received 2 August 2013, revised 2 December 2013
Accepted for publication 16 December 2013
Published 13 February 2014

*New Journal of Physics* **16** (2014) 025003
doi:10.1088/1367-2630/16/2/025003

Abstract

We investigate experimentally the emergence of collective motion in the bulk of an active suspension of *Escherichia coli* bacteria. When increasing the concentration from a dilute to a semi-dilute regime, we observe a continuous crossover from a dynamical cluster regime to a regime of ‘bio-turbulence’ convection patterns. We measure a length scale characterizing the collective motion as a function of the bacteria concentration. For bacteria fully supplied with oxygen, the increase of the correlation length is almost linear with concentration and at the largest concentrations tested, the correlation length could be as large as 24 bacterial body sizes (or 7–8 when including the flagella bundle). In contrast, under conditions of oxygen shortage the correlation length saturates at a value of around 7 body lengths.

Online supplementary data available from stacks.iop.org/NJP/16/025003/mmedia

1. Introduction

Large-scale collective and synchronized motion is present not only in animal groups [1, 2] and human crowds [3], but also in driven granular matter [4–7], dense actine networks [8] or active emulsions [9]. However, its origin and significance may stem from physically different origins and remain to a large extent an open issue. For animal motion an early line of thought relates the origin of collective motion to local interactions that favor the adjustment of individual motion...
on the mean value of its local surroundings (a gregarious tendency) [10]. Such models were studied theoretically and numerically [10, 11] and it was shown that large-scale flocking motion appears spontaneously as soon as the local gregarious tendency overcomes the orientation noise. This may lead to a rich variety of self-organized patterns [12]. In simple numerical simulations, noise is often taken as an external control parameter; however, in real systems the origin of local ordering and internal fluctuations remains unclear (mechanical or hydrodynamic fluctuations, perception, reaction capacities for living species, etc) [13]. These models essentially ignore the presence of a fluid background with the exception of a passive damping of particle motion. For suspensions of swimming microorganisms hydrodynamic theories were developed either based on the macroscopic transport equations phenomenology [14–16] or directly coming from a kinetic theory accounting explicitly for the transfer of momentum to the fluid and the hydrodynamic interactions between the swimmers [17, 18]. Numerical simulations of swimmers in a surrounding fluid were also performed to model active suspensions with the aim to test the theoretical predictions or to expand the studies to limits where the analytical theory is difficult [19–21]. In particular, the issue of collective organization of swimmers was addressed beyond the linear stability analysis of hydrodynamic modes [19, 20, 23]. An important outcome of these studies is that, for rear-activated swimmers or ‘pushers’ (as considered in this paper), long-range nematic ordering as well as fully isotropic states are both unstable and essentially lead to collective motion [14, 23]. The onset of collective motion may [17] or may not [18] depend on the system size [14, 15, 17, 22, 23].

From the experimental point of view, several studies were performed for self-propelled swimmers using bacteria [24], algae [27, 28] or self-propelled colloids [9, 29]. Unique physical phenomena caused by the activity of swimmers were identified such as enhanced Brownian diffusivity [24, 27, 30, 31], uncommon viscous response [28, 32, 34], active transport and mixing [35] or work extracted from the bacteria swimming activity [36, 37]. For bacteria strains such as Bacillus subtilis or paramecia, different authors reported large-scale collective motion baptized at the occasion of bio-turbulence [39, 42, 44–46]. Recently, a hydrodynamic equation was proposed for describing this regime [42, 43, 46]. However, it is probable that such large-scale motion is strongly influenced by the presence (or absence) of oxygen [41] or any other chemical species influencing the motility of the organisms as well as, in some cases, gravitational geotaxic effects [44]. However, B. subtilis were also reported to move collectively on agar plates in a dense swarm mode where obviously hydrodynamic interactions are absent [38]. For high concentrations the elongated aspect ratio is generally believed to play an important role in the global organization [47]. A similar observation has been made for passive non-biological systems such as mechanically activated rods [5, 6]. The anisotropic shape of the B. subtilis bacteria might also be at the origin of collective motion in the case of strong confinement in thin films where a sharp transition to collective motion as a function of the bacteria concentration was reported in a rather dense regime [40].

In this paper, we investigate the onset of collective motion in suspensions of Escherichia coli bacteria [48] in the bulk. E. coli are far less sensitive to oxygen shortage than B. subtilis and have a less elongated shape. We can thus expect hydrodynamic interactions to be more important for E. coli bacteria compared to B. subtilis and the study of their collective behavior might give additional insight in the underlying mechanisms. Some experimental studies using E. coli bacteria addressed the role of hydrodynamic interactions, but no systematic study of the emergence of large-scale structures for suspensions of E. coli bacteria in the bulk exists so far. Note, however, that in quasi-two-dimensional thin films, Wu et al [24] reported the spontaneous
organization of \( E. \ coli \) bacteria to form ‘swirls and jets’, but no direct quantitative measurement of this effect was made.

Drescher \textit{et al} \cite{33} explicitly determined the leading hydrodynamic parameters (local hydrodynamic field and effective force dipole parameter values) for \( E. \ coli \) bacteria. Consequently, long-range hydrodynamic effects would be particularly important for advection, the flow field scaling as \( 1/r^2 \) (\( r \) is the distance from the swimmer), whereas reorientations would essentially depend on vorticity scaling as \( 1/r^3 \), thus being more effective at shorter range. They suggest that steric effects also play a role in the alignment of bacteria, which will be clearly a dominant effect for higher concentrations. Wu \textit{et al} \cite{25} also performed a three-dimensional (3D) tracking of trajectories of \( E. \ coli \) bacteria at small concentrations, in a range comparable to the concentrations tested in the present paper, and state that hydrodynamic interactions between neighboring bacteria led to an increase in the diffusion coefficient. Using the same technique, Qian \textit{et al} \cite{26} studied pair velocity correlation for two given concentrations, again pointing out the importance of hydrodynamic interactions.

In a recent numerical contribution, Saintillan and Shelley \cite{23} studied the emergence of coherent structures and correlated dynamics solely caused by hydrodynamic interactions in active suspensions. They showed a progressive increase of the velocity correlation lengths with concentration when going from the dilute to the semi-dilute regime. The role of hydrodynamic interaction was also put in perspective in a contribution by Sokolov \textit{et al} \cite{41} who studied the collective organization of \textit{Bacillus subtilis}. These authors controlled the concentration of \( O_2 \) to vary both the swimming speed and the tumbling rate. They highlighted two fundamental mechanisms: long-range hydrodynamic interactions and close-range collisions. \textit{A priori} for microorganisms, collisions would include both hydrodynamic and steric interactions. They also identified a time scale (here the tumbling frequency) defining the coherence time of the collective structures.

The aim of the present work is to carry out quantitative measurements of the emergence of collective motion in a suspension of \( E. \ coli \) bacteria by systematically varying the concentration from the dilute to the semi-dilute concentration regime. We also investigate the influence of oxygen shortage on the collective behavior. We will compare our results to the existing theoretical predictions to get insight into the underlying mechanisms of the emergence of collective behavior.

2. Materials and methods

2.1. Experimental setup

We use wild-type \textit{E. coli} bacteria prepared following the experimental protocol described in \cite{31, 49}. The strain was grown overnight in lysogeny broth (LB). After washing, it was transferred into MMAP, a minimal medium supplemented with K acetate (0.34 mM) and polyvinyl pyrolidone (PVP: 0.005%). Then it was incubated for at least an hour in that medium. To avoid bacteria sedimentation, Percoll was mixed with MMAP (1 vol/1 vol) to obtain density matching between the bacteria and the medium. In this minimal medium, bacteria do not reproduce and they swim at a speed of around 20 \( \mu \text{m s}^{-1} \) \cite{34}. Chemicals inducing chemotactic driving are absent in the suspending medium except for those that might be produced by the bacteria themselves. For the rather dilute suspensions we use here, we may expect that the effect of such a signal is negligible with respect to hydrodynamic forces; however, it is quite
difficult to completely rule out this possibility when bacteria start to aggregate. The suspensions were prepared with the number of bacteria per unit volume \( n \) in the range \( 0.2 \times 10^{12} \text{ l}^{-1} < n < 80 \times 10^{12} \text{ l}^{-1} \). The bacteria body volume being taken as \( v_b = 1 \text{ \mu m}^3 \), this corresponds to a volumetric concentration \( \phi = n v_b \) in the range \( 0.02\% < \phi < 8\% \). With the \textit{E. coli} body length being \( a = 2 \text{ \mu m} \) the total length of the bacterium, taking also the flagella into account, can be estimated as \( l \approx 3–4a = 6–8 \text{ \mu m} \). In this case, the excluded volume fraction is \( \nu = \phi * (l/a)^3 \) and for \( \phi = 8\% \) we reach a value \( \nu = O(1) \). At a concentration of \( \phi = 8\% \) a semi-dilute regime is thus reached. When necessary, in order to monitor the flow velocity, a very low concentration of \( 2 \text{ \mu m} \) density-matched latex beads are suspended as passive tracers into the suspensions.

To contain the suspension, different types of geometries are used: rectangular PDMS channels of height \( h = 100 \text{ \mu m} \) and width \( W = 600 \text{ \mu m} \) (see figure 1(a)) and circular chambers of diameter \( 5 \text{ mm} \) made from a droplet of the suspension confined between two glass cover slips maintained at a distance of \( h = 100 \text{ \mu m} \) by two spacers (see figure 1(b)). The suspensions of bacteria are flown into the microfluidic channel, whereas the droplet of the suspension is directly deposited onto the cover glass. Furthermore, rectangular micro-fluidic channels are made in PDMS, a material known to be transparent to oxygen fluxes (avoiding in this way oxygen shortage for the bacteria), whereas in the droplet, oxygen can permeate through the droplet interface with air but not from the upper and lower edges. The PDMS channels are made using standard soft-lithography techniques. For the rectangular channels used in the experiments with and without flow, the inlets are connected by \( 500 \text{ \mu m} \) diameter tubes to a syringe pump. All experiments are performed at a fixed temperature of \( T = 25^\circ\text{C} \).

The bacteria suspensions are monitored using an inverted microscope (Zeiss-Observer, Z1). Bacteria moving in the flow are visualized with a high-magnification objective (100 \( \times \), phase contrast) allowing observation of the bacteria at various heights \( z \) (field depth 6.6 \text{ \mu m}). Videos were taken using a high-speed camera, Photron FastCam SA3, at resolution \( 1024 \times 1024 \text{ pixels} \), shutter speed 1/500 s and frame rate 125 fps.
2.2. Data analysis

First, the local bacteria velocities projected onto the $xy$ plane are obtained using a correlation-based PIV technique. The boxes used to access the motion correlation are of size 1.92 $\mu$m (i.e. 12 pixels), corresponding to the typical bacteria body length. The observation window is a square of size 135.2 $\mu$m. To process the image sequences, we use the commercial PIV software La Vision Davis 7.2.2. The correlation is done over a time step of 0.04 s. Note that for the typical swimming speed of the bacteria of 20 $\mu$m s$^{-1}$, the bacteria swim over a typical distance of 0.8 $\mu$m during the time interval of 0.04 s. This is much smaller than our field depth of 6.6 $\mu$m. Even bacteria swimming perpendicular to the $x$–$y$ plane do thus not leave the field of observation during this time. This means that we are essentially measuring projections of 3D trajectories. After processing, we obtain at each time step a spatially resolved velocity field $\vec{V}(\vec{r}, t)$ at time $t$.

We also analyzed the image sequences using a tracking algorithm developed in the group. In this case, the bacteria appearing as a white spot surrounded by a dark edge can easily be tracked at high frame rate if the concentration is not too high. See figure 1(b) and video 1 in the supplementary data (available from stacks.iop.org/NJP/16/025003/mmedia) for an animation of the bacteria tracking. The effective depth of field is here 3.4 $\mu$m. The difficulty is that most of the bacteria stay only fractions of a second in the field, so the lag time during which a bacterium is tracked can act as a filter on the population. For these results, we used the corresponding PIV lag time of 0.04 s. We have checked that varying the lag time slightly did not lead to a modification of the velocity correlations. The two methods (PIV and tracking) can be seen as complementary methods bearing their own possible limits and artifacts. Note that tracking becomes difficult at higher concentrations and higher flow rates. So for each claim we make in this paper, we tried to perform a crosscheck using both methods.

3. Experimental results

In this part, we will consider suspensions of bacteria observed at mid-height between the top and bottom walls of the chambers. Visual observation of the velocity fields clearly shows large-scale coherent structures (see figure 2). In figures 2(a)–(c), we represent for different packing fractions ($\phi = 0.1, 1$ and 2%) the spatial distribution of the velocity fields and in figures 2(d)–(f) a color map of the corresponding velocity directions. We can directly observe clusters of bacteria moving collectively in a given direction. Note that for the representation, displacements smaller than 1 px per 5 frames are not shown. See video 2 in the supplementary data for an animation of these cluster dynamics using the tracking method. In figure 3 the spatial velocity distribution is shown for a higher concentration ($\phi = 8\%$), corresponding to the semi-dilute regime. We clearly see large-scale collective motion resembling qualitatively the bio-turbulence patterns reported for $B. subtilis$ (see video 3 in the supplementary data).

To quantitatively analyze these structures, we calculated two types of spatial correlation functions: velocity correlation functions and angle correlation functions. The velocity correlation functions $C_{ij}(R) = \langle (V_i(\vec{r}) V_j(\vec{r} + \vec{R}) - \overline{V_i(\vec{r})^2})/\overline{V_i(\vec{r})^2} \rangle$ are computed as a function of the distance $R = ||\vec{R}||$, the indices $i, j$ represent the $x$ or $y$ direction. For each image, the correlation function is calculated as a spatial average (over-line) and a second average is taken over time (bracket) which corresponds to 4000 images. Similarly, we computed the
angular correlations using the velocity director $\vec{n} = \vec{V} / \|\vec{V}\|$ to monitor whether bacteria move in the same direction independently from their velocity amplitude. With the same definitions for the averages: $C_{\alpha}(R) = \langle \vec{n}(\vec{r})\vec{n}(\vec{r}+\vec{R}) - \vec{n}(\vec{r})^2 \rangle$. In figures 4(a) and (c), we display $C_{xx}$ and $C_{\alpha}$ for four values of the bacteria concentration $\phi$. The velocity correlations obtained from PIV and bacteria tracking show that the spatial organization of the bacteria varies with
concentration. Using an exponential fit we extract the values for the corresponding correlation lengths $\Lambda$ displayed as a function of $\phi$ in figures 4(b) and (d). When comparing the different methods (tracking/PIV and velocity correlation/angular correlation) or the different systems (channel/droplet) we obtain consistent results at concentrations $\phi < 2\%$. The correlation length increases in a quasi-linear way with the concentration.

For higher concentrations bacteria tracking becomes more difficult and we only display results obtained from PIV. In the microfluidic PDMS channel, we observe an increase of the correlation lengths with the concentration up to $\phi = 8\%$, the largest concentration tested. No saturation is observed. At $\phi = 8\%$ $\Lambda = 48 \mu m$ and the ratio $\Lambda/a \approx 24$ or $\Lambda/l \approx 8$, taking also the flagella into account. This is in agreement with previous observations for other coherent structures, where typical mesoscopic lengths up to ten bacterium sizes, including the flagella, were observed [39, 41–43, 45, 46]. Note that $\Lambda_{yy}$ (not shown) is identical to $\Lambda_{xx}$ for all concentrations. Importantly, the increase of the correlation length seems progressive and no apparent threshold for the onset of collective motion is observed.

Note that these results are qualitatively similar to the numerical simulations of Saintillan et al [23], who studied the collective behavior of slender rod-like pushers in the dilute and semi-dilute regimes. They also found a quasi-linear increase of the correlation length with concentration. However, it is important to note that these 3D numerical simulations were
performed in boxes of relatively small sizes: the box size $L$ is about ten times the bacterial body length $l$ and the authors show that the correlation length depends on the box size by slightly varying the latter. In our case, considering a bacterium total length of about $l = 6 \mu$m (including flagella) the vertical direction of the channel corresponds to about 17 bacteria lengths (flagella included) and a quantitative comparison of the results is thus difficult.

The experimental results become very different when the bacteria are confined between two cover glass slides. No oxygen can diffuse inside through the glass slides. Interestingly, in this case, we observe a saturation of the correlation length at a value of $\lambda = 14 \mu$m. This might indicate that at higher concentration, oxygen shortage might become important, leading to a saturation of the correlation length, as observed by Sokolov et al [41].

4. Summary and conclusion

In this paper, we show the progressive emergence of collective motion in the bulk of a suspension of *E. coli* bacteria when the concentration is increased from the dilute to the semi-dilute regime. In the small concentration regime, we observe cluster-like structures as isolated patches of bacteria moving in the same direction. The formation of these clusters may be due to an interplay between the hydrodynamic interactions and the collisions producing a subsequent ordering of the swimmers. At the higher concentrations, we observe the emergence of large-scale coherent motion similar to the bio-convection patterns reported earlier for *B. subtilis* [39, 41–43, 45, 46]. The velocity and orientation correlation functions were measured and the correlation lengths were extracted. We show a progressive and almost linear increase of the correlation length with concentration up to large mesoscopic scales. Note that these results are qualitatively similar to the numerical results of Saintillan and Shelley [23] whose simulations solely take into account the hydrodynamic interactions between swimmers. Interestingly, we observed a saturation of the correlation length with concentration when the bacteria are deprived of oxygen. These results point out the importance of a coherence time scale limiting the spatial extension of collective motion [41]. In practice, this time scale may be fixed by the tumbling frequency which is enhanced for *E. coli* in the case of oxygen reduction [50], a result consistent with the observations of Sokolov et al [41] for *B. subtilis*.

The existence of collective motion in such dilute or semi-dilute suspensions of bacteria may have an important influence on the macroscopic behavior of these suspensions and might have to be taken into account for further modeling the rheology of these suspensions in the semi-dilute regime [34].

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