Fluid dynamics of bacterial turbulence

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Self-sustained turbulent structures have been observed in a wide range of living fluids, yet no quantitative theory exists to explain their properties. We report experiments on active turbulence in highly concentrated 3D suspensions of Bacillus subtilis and compare them with a minimal fourth-order vector-field theory for incompressible bacterial dynamics. Velocimetry of bacteria and surrounding fluid, determined by imaging cells and tracking colloidal tracers, yields consistent results for velocity statistics and correlations over two orders of magnitude in kinetic energy, revealing a decrease of fluid memory with increasing swimming activity and linear scaling between energy and enstrophy. The best-fit model parameters allow for quantitative agreement with experimental data.

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A series of experiments over the last decade [1–10] has shed light on generic ordering principles that appear to govern collective dynamics of living matter [11,15], from large-scale animal swarming [1,2] to meso-scale turbulence in microbial suspensions [3,8] and micro-scale self-organization in motility assays [9,10]. Although very different in size and composition, these systems are often jointly termed ‘active’ fluids, for which there is now a range of continuum theories [12–22]. From these have come important qualitative insights into instability mechanisms [13–16,20,23] driving dynamical pattern formation, but a quantitative picture remains incomplete: even for the simplest active (e.g., bacterial or algal) suspensions uncertainty remains about which hydrodynamic equations and transport coefficients [24,25] provide an adequate minimal description, due in large part to the inability of existing data to constrain the manifold parameters in these models. One approach to remedy this problem is to characterize collective dynamics as in high Reynolds number fluid turbulence, in terms of kinetic energy, enstrophy and spatio-temporal correlation functions, and to compare with an appropriate long-wavelength theory (i.e. Navier-Stokes-type equations). We present such an analysis here, measuring collective behavior in dense suspensions of the bacterium Bacillus subtilis in comparison to predictions of a (fourth-order) continuum model for bacterial flow [7,26].

Previous experimental studies of bacterial suspensions in open droplets [3,4,27,28], freestanding films [5,8,25,29], on surfaces [6,30,31], or quasi-2D microfluidic chambers [7] focused separately on the bacterial and fluid components, leaving uncertain how accurately passive tracers [32,33] reflect collective bacterial dynamics. The experiments reported here, performed in closed 3D microfluidic chambers, allowed near-simultaneous measurements of cell and tracer motion, and exploit a natural reduction in bacterial swimming activity due to oxygen depletion [8,27,29] to obtain data spanning two orders of magnitude in fluid kinetic energy. Combined with extensive 3D numerical simulations of the model, this data allows robust parameter estimates. Quantitative agreement between experiment and theory suggests that this model presents a viable generalization of the Navier-Stokes equations to incompressible active fluids.

Wild-type strain 168 of B. subtilis has cigar-shaped cell bodies on average 0.8 µm in diameter and 5 µm long [7]. It was streaked on LB medium agar plates from frozen stocks. Colonies from these plates were used to inoculate overnight cultures in Terrific Broth (TB; Sigma), which were back-diluted 1:100 into 100 ml of TB and grown to mid-logarithmic phase on an orbital shaker at 37°C. These cultures were then concentrated 400- to 4,000-fold (final volume fraction ∼0.5%), and fluorescent microspheres (diameter 1 µm, F-8816, Invitrogen) were added at a final concentration of ∼10^9 beads/ml. The resulting suspensions were loaded into polydimethylsiloxane (PDMS) microfluidic devices, consisting of a series of cylindrical chambers (radius 750 µm, height 80 µm), connected by thin channels [7,50]. The inlet and outlet of the device were sealed with vacuum grease, and images were acquired in the (xy)-midplane of the chambers, ∼40 µm above the bottom, using a Zeiss 40× (NA 1.3) oil immersion objective and a high-speed camera at 40fps (Fastcam SA-3, Photron). Movies were recorded in pairs for each field of view (768 × 800 pix; 1 pix = 0.36 × 0.36 µm^2), one with bright-field illumination and one with fluorescence excitation by a 633 nm laser (B&W Tek) at ∼20 mW. These movies were taken immediately after each other with a ∼3 min time lag between subsequent pairs. During the ∼10 min imaging period for each
While the vertical component of vorticity and kinetic energy evolve, movie segments (40 fps, each 50 s long) corresponding to the bacterial flow velocity (Fig. 1). Data were analyzed under the assumption that they are passively advected. Data shown in Figs. 2 and 3 are based on 7 movies (40 fps, length 100 s) at different activities. Trajectories of individual tracer particles were found with a custom algorithm which, depending on seeding density and tracer dynamics, was able to identify up to 10 individual tracks, the longest typically lasting 5 – 8 s. The effective sample size was insufficient to determine reliably the tracer VACFs, but did yield global flow properties, velocity histograms and equal-time VCFs. The velocity PDFs, calculated directly from individual tracer velocities, are approximately Gaussian with a peak at small velocities from tracer accumulation near the vortex centers (Fig. 2b).

Estimates from PTV for the medium VCF and enstrophy were obtained by interpolating tracer velocities on a 450 × 450 pix subwindow in the center of the imaging plane using MATLAB’s Delaunay triangulation with a lattice spacing $\Delta = 90 \sqrt{\text{pix}/N_f}$, where $N_f$ is the mean number of tracers detected per frame. The accuracy of this reconstruction procedure is controlled by the tracer concentration, which was kept low to limit effects on the bacteria motion and to avoid tracking ambiguities (typically $N_f \in [47, 144]$ for data shown in Figs. 2 and 3).

As a result, the uncertainties for the PTV data are considerably larger than for PIV data (see Fig. 2l). The interpolated tracer flow fields were used to estimate the kinetic energy $E_{xy}$, enstrophy $\Omega_z$, and spatial correlation functions of the in-plane medium flow components.
In agreement with the PIV results for the bacterial flow, we find again a linear enstrophy-energy relation (Fig. 2d) and comparable vortex radii, using the first zero of VCF as an estimate (Fig. 3a). We may therefore conclude that, at our very high bacterial concentrations, solvent and bacterial flow statistics become tightly linked.

We now examine how these data compare to predictions of a theory of active fluids introduced recently [11]. This minimal continuum model assumes that, at intermediate concentrations [28], the parameter $\lambda_0$ describes advection and nematic interactions, and $\lambda_1$ an active pressure contribution [26]. For pusher swimmers like $B$. subtilis, general considerations of hydrodynamic [12] and nematic stresses [26, 43] suggest that $\lambda_0 \geq 1$ and $\lambda_1 \approx (\lambda_0 - 1)/3 \geq 0$ in 3D. The $(\beta, v_0)$-terms correspond to a quartic Landau-type velocity potential [14, 18, 40] and are physically motivated by the observation of extended jet-like streaming regions in $B$. subtilis suspensions at intermediate concentrations [28]. The parameter $v_0$ defines the collective speed that would be achieved if all bacteria were to move in the same direction. When $\beta \neq 0$ the model does not conserve momentum or energy, which may exchange energy and momentum with the solvent. The nonlocal $(\Gamma_0, \Gamma_2)$-terms encode passive and active stresses due to hydrodynamic and steric interactions. For $\lambda_0 = 1$, $\lambda_1 = \beta = \Gamma_2 = 0$ and $\Gamma_0 > 0$, the model reduces to the incompressible Navier-Stokes equation. A detailed stability analysis [26] shows that when $\lambda_0 \neq 0$, $\beta > 0$, $v_0 > 0$, $\Gamma_2 > 0$ and $\Gamma_0 < 0$ this is one of the simplest vector models to describe phenomenologically the formation of jets and turbulent vortices in quasi-incompressible active suspensions. Very recently, the 2D version of Eq. (1) has been shown to provide a quantitative mean field description of bacterial meso-scale turbulence in quasi-2D suspensions [7]. Its applicability to the physically more relevant 3D case is first explored here.

We simulated Eq. (1) in 3D with periodic boundary conditions using a pseudospectral operator-splitting algorithm [44, 45] and a pressure correction subroutine to ensure incompressibility [7, 26]. Simulation grids ranged from $128^3$ lattice points for parameter pre-screening to $256^3$ for statistical analysis. Numerical stability of the solver was verified for a wide range of parameters and
space-time discretizations. All simulations were initiated with randomly chosen velocities. Figure 4 shows structure-formation in a typical simulation domain.

Since in 3D we have \( \lambda_1 \approx (\lambda_0 - 1)/3 \) [20], Eq. (1) has essentially five free parameters \((\lambda_0, \beta, v_0, \Gamma_0, \Gamma_2)\). Two of those can be eliminated by choice of appropriate length and time units. We adopt a natural unit system such that the vortex wave-length scale \( \Lambda_T = 2\pi \sqrt{\Gamma_2/(-\Gamma_0)} = 2\pi \) and \( v_0 = 1 \). In our simulations, the box length is fixed as \( L = 12\Lambda_T \), corresponding to approximately twice the experimental field of view, and the time step as \( \Delta t = 0.05\Lambda_T/(2\pi v_0) \). To estimate the three remaining parameters \((\lambda_0, \beta, \Gamma_0)\), we note that \( \Gamma_0 \) and \( \Gamma_2 \) define a typical vortex speed \( V_T = \sqrt{-\Gamma_0/\Gamma_2} \). In the turbulent regime, it is plausible that \( V_T \) is smaller than but close to \( v_0 \), i.e. \( V_T = \zeta v_0 \) where \( \zeta \ll 1 \). Furthermore, for pushers, the dimensionless parameter \( \lambda_0 \) should be larger than 1, but smaller than for quasi-2D suspensions [21], since nematic (steric) stresses can be more easily avoided in 3D; we infer \( \lambda_0 \sim 2 \). Finally, the acceleration time scale \( \tau_0 = (\beta v_0^2)^{-1} \) should be of the order of the vortex time-scale \( \Lambda_T/V_T \). Using these estimates as initial values in a systematic parameter scan, and by comparing with the bacterial PIV data, we obtained the best-fit parameters in Table I. Generally, the VCFs and VACFs respond sensitively to parameter variations in the simulations, suggesting that the estimates in Table I are accurate within 10-15% for quasi-incompressible \( B. \) subtilis suspensions. As an independent cross-check, we computed \( \Lambda = (E_{xy}/\Omega_z)^{1/2} \) from the best-fit simulation using \( \Lambda_T \sim 50 \mu m \) and found \( \Lambda \sim 29 \mu m \) which compares well with the experimental PIV value in Fig. 2a. We stress that the conserved form of the bacterial velocity PDFs (Fig. 2a), VCFs, and VACFs (Fig. 3) implies that all our experiments can be fitted by a single set of rescaled parameters \((\lambda_0, \beta, \Gamma_0)\), as it suffices to adjust the physical values of \( v_0 \) and \( \Lambda_T \) to match the kinetic energy and vortex length at a given bacterial activity level. As evident from the flow patterns in Fig. 1 and from the solid curves in Figs. 2a and 3, the best-fit parameters yield good qualitative and quantitative agreement with the experiments.

For incompressible ‘passive’ fluids, that are governed by the Navier-Stokes equations transport parameters have of course been measured for a wide range of materials [17]. In contrast, quantitative theories of even the simplest active fluids have been lacking. We have shown here that the minimal fourth-order vector model [17, 20] in Eq. (1) reproduces the main statistical features of self-sustained 3D bulk turbulence in concentrated bacterial suspensions, suggesting that this theory is a viable candidate for the quantitative description of incompressible active fluids. Due to the close correlation between bacterial and medium (tracer) flow observed in our experiments, we expect that this generic model will be useful in a wide range of future applications, in particular for predicting the effects of confining geometries on collective microbial dynamics [47, 48] and for understanding the anomalous viscosities of active fluids [24, 25].

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### Table I: Parameters of the best-fit continuum model

| model parameter        | in rescaled units | in physical units |
|------------------------|-------------------|-------------------|
| \( \Lambda_T = 2\pi \sqrt{\Gamma_2/(-\Gamma_0)} \) | \( 2\pi \) | \( \sim 50 \mu m \) |
| \( \lambda_0 \)         | 1.7              | 1.7               |
| \( V_T = \sqrt{-\Gamma_0/\Gamma_2} \beta \) | 0.9 \( v_0 \) | 0.9 \( v_0 \) |
| \( \varepsilon_{xy} \)   | 0.54             | 0.54 \( v_0^2 \) |

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