Mechanically Driven Growth of Quasi-Two-Dimensional Microbial Colonies

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We study colonies of nonmotile, rod-shaped bacteria growing on solid substrates. In our model, bacteria interact purely mechanically, by pushing each other away as they grow, and consume a diffusing nutrient. We show that mechanical interactions control the velocity and shape of the advancing front, which leads to features that cannot be captured by established Fisher-Kolmogorov models. In particular, we find that the velocity depends on the elastic modulus of bacteria or their stickiness to the surface. Interestingly, we predict that the radius of an incompressible, strictly two-dimensional colony cannot grow linearly in time, unless it develops branches. Importantly, mechanical interactions can also account for the nonequilibrium transition between circular and branching colonies, often observed in the lab.

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Active matter, which constantly takes energy from its environment in order to do work [1], has recently attracted much interest. Particular examples are collections of cells such as tissues and suspensions of swimming bacteria [2–4], and microbial colonies, in which activity is caused by growth, death, and migration of cells. The combination of these three factors has been shown to lead to a variety of interesting and universal patterns [5–8]. For example, bacteria such as *B. subtilis* or *E. coli* grown on Petri dishes form patterns ranging from circular, through Eden-like [9], to diffusion-limited aggregationlike patterns [10]. Such patterns have been traditionally modelled using a system of diffusive Fisher-Kolmogorov equations [11,12] which combine migration (diffusion of bacteria), bacterial growth, and nutrient diffusion. This approach, however, does not accurately represent the growth on surfaces on the microscopic level, where expansion is caused by cells pushing each other out of the way as they grow, rather than by migration.

In this Letter, we study the role of mechanical interactions in the growth of dense colonies on solid substrates. Inspired by recent experiments in microfluidic devices [13], we study quasi-two-dimensional growth of a colony of nonmotile single-celled organisms which consume nutrient in order to grow and divide. We argue—supported by computer simulations and analytical calculations—that mechanical interactions between bacterial cells can account for the emergence of a nonequilibrium transition between quasi-circular and branched colonies as a function of the ratio between the nutrient consumption rate and the growth rate. The strength of mechanical interactions determines the speed with which the colony expands in space, with diffusion of the nutrient playing a secondary role. We also show that the leading edge of the front is very sharp, and the bacterial density is discontinuous at the front, in contrast to a smooth, exponential profile predicted by models based on coupled Fisher equations [8,12]. Our results are relevant to the growth of biofilms [14–16], which are ubiquitous in nature and are involved in a variety of medical and technological problems. As

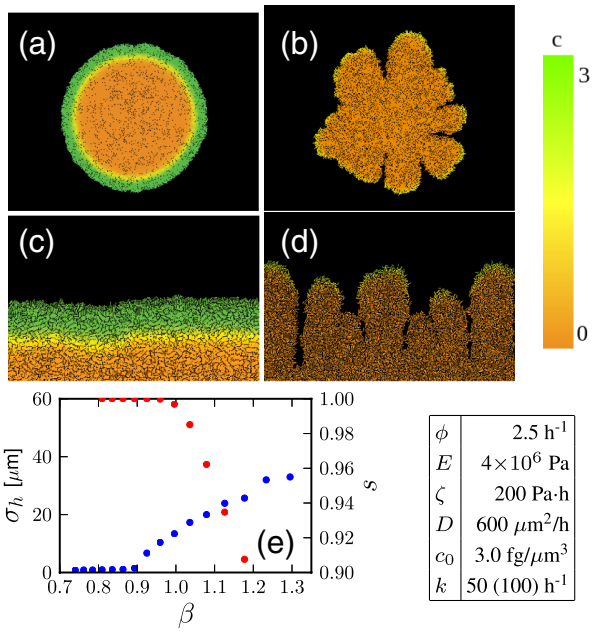

FIG. 1 (color online). (a),(b) Snapshots from the simulation of $N \sim 10^5$ cells, for low (a) and high (b) values of the branching parameter β (see also, videos in Supplemental Material [19]). Colors correspond to the local nutrient concentration, see the color map on the right. Only a thin layer of cells grows appreciably. (c),(d) Growth in a narrow, long strip, for low (c) and high (d) β. The frame is comoving with the front. (e) Roughness of the front σ_h (blue or dark points) and fraction of space filled by cells, s (red or light points), as a function of β (flat geometry, box width $L = 250$ μm). Table shows parameter values used; k was increased to 100 in (b) and (d). These are the default parameter values used in the rest of the Letter unless otherwise stated.

mechanical interactions may alter the colony morphology, and the fixation probability of (potentially harmful) mutants [17,18], understanding their role is of paramount importance.

We simulate bacteria using two-dimensional Newtonian dynamics. Cells are modelled as growing spherocylinders of constant diameter $d = 1\ \mu$m and variable length that split in half to yield two cells when they reach some critical length ℓ_c (usually 4 μm). The colony grows on a two-dimensional flat surface with nutrient concentration $c(x, y)$. The nutrient diffuses with diffusion constant D. Initially, $c = c_0$ everywhere, and c is always held constant at the edges of the simulation box, which is made large enough that the boundary does not affect the growth. Nutrients are consumed at a rate $kf(c)$ per unit biomass density, where $f(c)$ is a monotonically increasing dimensionless function. In most simulations, we use a Monod function $c/(c_{\text{half}} + c)$ with half-saturation constant c_{half}. Cells grow (by elongation) at a rate $v_g f(c)$. All parameters and their values are detailed in the Supplemental Material [19].

The cells interact mechanically. The force between overlapping bacteria is assumed to be given by the Hertzian theory of elastic contact [13,20,21]: $F = Ed^{1/2}h^{3/2}$ where h is the overlap and E parametrizes the strength of the interaction and is proportional (modulo a dimensionless prefactor) to the elastic modulus of the cells, and the dynamics is overdamped so that velocity is proportional to force, $v = F/\zeta$, where ζ is the friction coefficient.

We start our simulations from either a single initial cell or a line of cells, and follow the shape of the colony after many rounds of cell replication, leading to a circular colony or a horizontal advancing front, respectively. Figure 1 shows that the morphology of a large colony of bacteria can be either smooth or branched, depending on the parameters of the model.

By performing simulations for different parameter sets we have found that the fate (smooth or branched) of the colony is determined by a dimensionless "branching parameter" $\beta = (k\rho_0)/(\phi c_0)$, where ρ_0 is the close-packed cell density, and the other parameters have been defined previously.

For small values of β, the front of the colony remains smooth throughout the simulation [Figs. 1(a) and 1(c)], whereas for large values branches develop [Figs. 1(b) and 1(d)]. Note that, as in real colonies [17], the nutrient becomes depleted within the colony so that only cells in a thin layer at the front are growing. To pinpoint the location of the transition, we compute the roughness of the front [Fig. 1(e)], defined as the mean square deviation of points on the front from its average position, as in Ref. [6]. At $\beta \simeq 0.9$, there is a transition from a flat to a rough front, whereas at $\beta \simeq 1$, there is a switch between a quasicircular front and one with branches, demonstrated by the filling fraction s falling below 1. This behavior is similar to that observed in Ref. [6].

This transition between branched and smooth colony fronts is well known in real colonies [22] and has been the subject of many theoretical studies [8,12], which usually attribute it to the interplay between diffusion (migration) of bacteria and diffusion of the nutrient. In our model, however, the transition is driven by the uptake of nutrient by the cells and their growth by mechanical pushing, and is unaffected by the diffusion rate of the nutrient.

To gain a better understanding of this transition, we approximate the growing colony as an incompressible cellular "fluid" [23]. Mass conservation in such a fluid is described by the equation $\nabla \cdot \mathbf{v} = \phi f(c(\mathbf{x}))$, where $\mathbf{v}$ is the fluid velocity, $f(c)$ is the dimensionless nutrient uptake function, and ϕ is the growth rate of the cellular fluid, given by $\phi = v_g/\ell_c$. Let us begin with a one dimensional case of a colony advancing from the left and consuming nutrient, and characterized by a single number $x_0(t)$ which is the position of the front

$$\partial_t c(x, t) = D\partial_x^2 c(x, t) - k\rho_0 f(c(x, t))\Theta(x_0 - x), \quad (1)$$

$$v(x_0) = \frac{dx_0}{dt} = \phi \int_{-\infty}^{x_0(t)} f(c(x, t))dx. \quad (2)$$

Here D is the nutrient diffusion constant, k the rate of uptake of nutrient by cells, ρ_0 the cell density (constant everywhere due to incompressibility), and Θ is the Heaviside step function. Because cells do not migrate and they are tightly packed, the density is either ρ_0 or zero, and hence, Eq. (2) can be derived from the continuity equation and the incompressibility condition, assuming that $\rho(x, t) = \rho_0\Theta(x_0(t) - x)$. We also impose boundary conditions that $c(-\infty) = 0$ and $c(\infty) = c_0$.

We first determine whether Eqs. (1) and (2) admit a travelling-wave solution $c(x, t) = \hat{c}(x - vt) \equiv \hat{c}(z)$ in the limit $t \to \infty$, where the velocity v of the front is constant. The resulting equations for $\hat{c}(z)$ and v are

$$-v\hat{c}'(z) = D\hat{c}''(z) - k\rho_0 f(\hat{c})\Theta(-z), \quad (3)$$

$$v = \phi \int_{-\infty}^{0} f(\hat{c}(z))dz. \quad (4)$$

For $z > 0$, the solution to Eq. (3) is given by $\hat{c}(z) = c_0 + Ae^{-vz/D}$ [as $c(\infty) = c_0$]. For $z < 0$, we can rearrange the equation to yield $f(\hat{c}(z)) = (1/k\rho_0)[D\hat{c}''(z) + v\hat{c}'(z)]$, which, upon insertion into Eq. (4), gives

$$v = \frac{\phi}{k\rho_0}[D\hat{c}'(0) + v\hat{c}(0)] = \frac{\phi c_0}{k\rho_0}v, \quad (5)$$

where we have integrated by parts, and used the fact that $\hat{c}$ vanishes at $-\infty$, and that $\hat{c}$ and $\hat{c}'$ must be continuous at $z = 0$. Therefore, a solution for v exists only if $\phi c_0 = k\rho_0$ (or $\beta = 1$) exactly: we have found that in the incompressible limit the front cannot advance at a constant speed. This is in contrast to the Fisher framework, where travelling waves exist for a range of parameters. Numerical solutions

of Eqs. (1) and (2) fully confirm our prediction, showing exponential growth for $\beta < 1$ and sublinear growth for $\beta > 1$, see Supplemental Material [19]. This is only true if the growth and the uptake rate on c have the same functional form, $f(c)$. This is a good approximation for *E. coli* and other bacteria with low maintenance costs [24], but is not true in general [25]. Choosing different dependencies on c for these rates, however, leads to qualitatively similar conclusions, with constant growth possible in a narrow window of β close to 1.

The hint from this simplified 1D model is that $\beta = 1$ is a critical value that separates different regimes of colony growth. For $\beta > 1$, growth is limited by nutrient diffusion, whereas for $\beta < 1$ diffusion does not play any role. However, the front has more freedom in 2D than in 1D—it can become branched. Since this transition occurs close to $\beta = 1$, it is appealing to conjecture that the branching transition in Fig. 1 is linked to the switch in growth laws for incompressible colonies described above.

Second, incompressible theory predicts that growth cannot be linear, unless $\beta = 1$ exactly. This is inconsistent with experimental results: the size of a colony of nonswimming bacteria growing on stiff agar gels does increase linearly with time [26]. Moreover, our simulations also lead to a finite steady state speed. The speed found in simulations depends on the elasticity E, as can be seen in Fig. 2(a), suggesting the compressibility of the cells is important.

Generalizing the theory above to compressible cells in 1D, we now need equations for mass and momentum conservation, as well as the nutrient diffusion equation

$$\partial_t c = D\partial_x^2 c - k\rho f(c), \tag{6}$$

$$\partial_t \rho + \partial_x(\rho v) = \phi\rho f(c), \tag{7}$$

$$\partial_x p = -\mu\rho v. \tag{8}$$

The term $\mu\rho v$ describes the friction between the surface and the cells. The pressure $p(\rho)$ is determined by the force acting between the cells. We take $p[\rho(x)] = E[1 - \rho_0/\rho(x)]^{3/2}$ to be consistent with our simulations, because the force that acts between two overlapping cells is then proportional to $Ed^{1/2}h^{3/2}$, where $h = d[1 - \rho_0/\rho(x)]$ is the overlap. ρ_0 is the uncompressed density of closely packed cells.

Although Eqs. (6)–(8) cannot be solved analytically, numerical solution (see Supplemental Material [19]) shows that a travelling wave now exists for $\beta < 1$. The density profile close to the edge decays according to a power law towards the uncompressed cell density ρ_0. This is in striking contrast to Fisher-Kolmogorov waves, which exhibit exponential density profiles in the wave tip [12]. Many other properties of the solution to Eqs. (6)–(8) can be deduced without solving the equations. First, a "biomass conservation law" from Eqs. (6) and (7) states that one unit of nutrient biomass makes ϕ/k units of bacterial biomass,

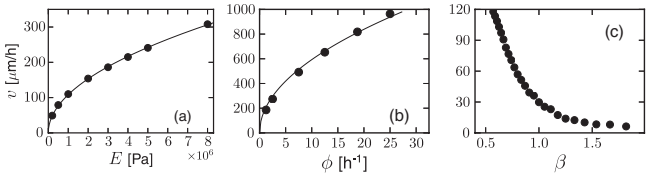

FIG. 2. Steady state speed of colony growth, v, as a function of various parameters, for 2D simulations in the quasi-1D geometry. (a) and (b) have fits to a square root function. In (b), ϕ is varied while holding β constant (by inversely varying k). (c) shows the dependence on β (c_0 is varied while keeping other parameters constant), with a change in behavior around $\beta = 1$.

and hence the density $\rho(-\infty)$ deep in the colony must be $\phi c_0/k$. This explains why a travelling wave solution cannot exist in the incompressible case: unless the cell density ρ_0 equals exactly $\phi c_0/k$ it will not match the density of biomass produced by the nutrient. It also explains why there is a morphological transition to branched colonies at $\beta \simeq 1$: growth of a compact colony is not possible for $\beta > 1$ as it would need to have a density less than ρ_0. Finally, it suggests that if bacteria are restricted to grow as a monolayer, then, when nutrient is abundant, they will grow exponentially until intermicrobial forces within the colony are so large that the bacteria in the middle are squashed to the appropriate density ρ_0/β.

We can estimate the velocity of the travelling wave using a simple scaling argument. At steady state, the cells are compressed to the strain $\epsilon \equiv 1 - \beta$, and the pressure profile has to rise from 0 at the edge of the population to a maximal value p_* in the bulk within a boundary layer of characteristic size λ. The characteristic length λ can be eliminated by estimating it to be the length by which the front moves in one generation $\lambda \approx v/[\phi f(c_0)]$. The bulk value of the pressure $p_*(\epsilon)$ is just large enough that the density of the population is compressed down to the strain ϵ. The elastic constitutive relation $p_*(\epsilon)$ of the microbial population fixes the corresponding pressure, with $p_*(\epsilon) = E\epsilon^{3/2}$ in our case of Hertzian contacts between cells. The pressure p_* pushes the front population at the speed v against the friction force $v\mu\rho_0\lambda$, where $\mu\rho_0$ acts as a friction coefficient per unit length. Thus, force balance yields

$$v \approx \sqrt{\frac{\phi f(c_0) p_*(\epsilon)}{\mu\rho_0}} = \sqrt{\frac{E\phi f(c_0)}{\mu\rho_0}} g(\beta), \tag{9}$$

where $g(\beta) = (1 - \beta)^{3/4}$.

To test the above formula, we performed a fully one-dimensional version of our simulations described above, as this removed the effects of branching and was much more computationally efficient. The results are shown in Fig. 3. Figure 3(a) shows that the front speed grows as $\sqrt{E}$ as predicted by Eq. (9), and Fig. 3(b) shows that the dependence of v on β is in good agreement with the numerically and theoretically predicted $g(\beta)$, although the theoretical

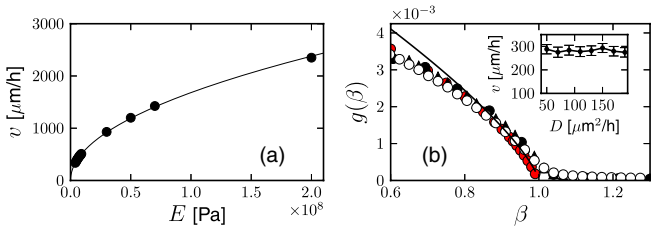

FIG. 3 (color online). Dependence of front speed on parameters in the fully 1D simulation. (a) Front speed as a function of elastic modulus E, with fit to $v = A\sqrt{E}$. (b) Transition from moving to stopped front as a function of β, which occurs when $\beta = 1$. $g(\beta)$ [defined by Eq. (9)] is plotted against β (by varying k) for $\phi = 10$ (open circles), 20 (triangles), and 30 (closed circles), showing a good collapse. Here $E = 4 \times 10^6$, $D = 100$. Solid line corresponds to theoretical $g(\beta) = (1 - \beta)^{3/4}$, and red (grey) circles are the numerical solution of Eqs. (6)–(8). Inset: v as a function of D, showing no dependence.

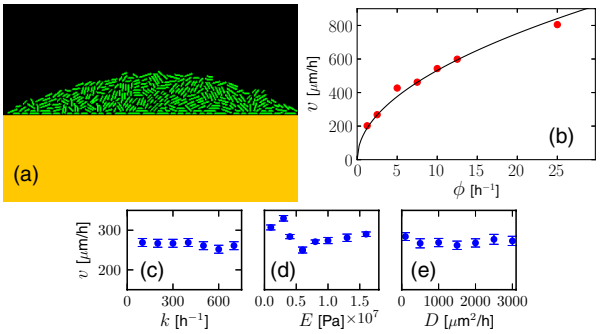

FIG. 4 (color online). Quasi-3D colony growth. (a) Snapshot. (b) Speed of radial colony growth against ϕ, with fit to $A\sqrt{\phi}$. (c)–(e) Speed against k, E and D, showing little dependence on any of these parameters. Parameters not being varied take their default values (Fig. 1).

form $g(\beta) = (1 - \beta)^{3/4}$ is only accurate for β close to 1. Figure 2 shows that the square-root dependence on E and ϕ also holds in the 2D case, but the function $g(\beta)$ is again different, and does not go to zero for $\beta > 1$, due to the branching. In the Supplemental Material [19], we perform a more rigorous derivation of Eq. (9), showing that it is valid when the dimensionless parameter $G = E/(\mu D \rho_0) \gg 1$ and β is close to 1. We also show that mechanics-dominated growth $G \gg 1$ is relevant for any experimentally feasible parameters. An interesting feature of this limit is that, since the dynamics are dominated by mechanics rather than nutrient diffusion, v does not depend on D.

So far, our findings are relevant to bacteria growing in monolayers. On agar plates, however, cells are observed to build up vertically in the colony center [27,28]. To probe how this affects our results, we simulate a colony growing in a vertical 2D plane xz (where the z axis is perpendicular to the substrate) instead of the xy plane from previous simulation. We also incorporate attractive cell-substrate interactions, and we model the diffusion in the $z < 0$ half-plane only, which models the substrate. This situation is far more computationally efficient than fully 3D simulations (see Supplementary Material [19]), and still allows us to study the effect of vertical growth. As is apparent from Fig. 4(a), cells do now escape out of the plane they start from, due to the force exerted by neighbors. The size of the colony once again grows linearly in time. However, it is not compressibility, but the possibility of escape into the vertical direction, which leads to linear growth.

In fact, if the bulk pressure $p_*(\epsilon)$, which builds up in a strictly two-dimensional setting, is larger than some critical pressure p_c, cells will escape into the z dimension. As a consequence the pressure profile will saturate at p_c in the bulk of the population. In our scaling argument for the speed of the front growth, we then have $v \approx \{[\phi f(c_0) p_c]/(\mu \rho_0)\}^{1/2}$. Figures 4(b)–4(e) show that, in contrast to the 2D case, the expansion speed $v \sim \sqrt{\phi}$ is now independent of the consumption rate k, elastic modulus E, and the diffusion constant D. Note that while the radial growth is independent of k, the vertical growth will be affected by it.

In conclusion, we have studied the growth of bacterial colonies where nonmotile microorganisms replicate and push each other away as they grow. We find a transition between two different growth regimes, controlled by the balance between growth and uptake of nutrients. Our model differs from biofilm simulations [29,30] which do not explicitly model mechanical forces. We also find that the functional form of the density profile close to the bacterial edge qualitatively differs from those predicted by Fisher-Kolmogorov models, and predict that the speed at which the front propagates depends only weakly on the nutrient diffusion rate D, for a wide range of D. Our predictions should be experimentally testable, especially in 3D, or directly in 2D using a microfluidic device restricting cell growth to a single layer. This could be used to estimate the elastic modulus of the cells through Eq. (9).

Additionally, our results may be relevant in other situations involving the growth of cells under limiting conditions, such as animal and cancerous tissues, which similarly involve a collection of cells proliferating and pushing on each other as they grow, often with their growth limited by the diffusion of nutrients. Mechanical interactions are understood to be very important in such systems; in particular, mechanical pressure has been hypothesized to strongly affect the growth and apoptosis rates of cells, leading to an alternative form of growth limitation [31–33]. Simulations and experiments indicate that this can lead to a steady state speed of growth [31]. It would be interesting to model this effect in our framework, and to study its interplay with nutrient limitation of growth.

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