Extinction transition in bacterial colonies under forced convection

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We report the spatiotemporal response of *Bacillus subtilis* growing on a nutrient-rich layer of agar to ultraviolet (UV) radiation. Below a crossover temperature, the bacteria are confined to regions that are shielded from UV radiation. A forced convection of the population is effected by rotating a UV radiation shield relative to the Petri dish. The extinction speed at which the bacterial colony lags behind the shield is found to be qualitatively similar to the front velocity of the colony growing in the absence of a hostile environment as predicted by the model of Dahmen, Nelson, and Shnerb. A quantitative comparison is not possible without considering the slow dynamics and time-dependent interaction of the population with the hostile environment.

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Bacterial colonies growing on a nutrient-rich substrate have served as model systems for studying pattern formation and population dynamics in biological systems. Studies with strains of *Bacillus subtilis* and *Escherichia coli* have reported a wide variety of complex patterns depending on nutrient conditions [1–4]. The patterns have been modeled using reaction-diffusion equations [5–7]. These experimental and theoretical studies have considered an essentially uniform environment where the changes are due only to the depletion of nutrients with time. However, living organisms often are forced to migrate due to changes in the environment.

The modeling of population dynamics of bacterial colonies due to changes in the environment has been studied recently by Nelson, Shnerb, and Dahmen [8,9]. Their theoretical model incorporates the effect of a forced convection on the growth of a bacterial colony by considering the convective-diffusion equation given by

$$\frac{\partial c(\mathbf{x},t)}{\partial t} = D\nabla^2 c(\mathbf{x},t) - \mathbf{v}\cdot\boldsymbol{\nabla} c(\mathbf{x},t) + U(\mathbf{x})c(\mathbf{x},t) - bc^2(\mathbf{x},t), \tag{1}$$

where $c(\mathbf{x},t)$ is the bacteria number density, D is the diffusion constant of the bacteria, $U(\mathbf{x})$ is the spatially varying growth potential, $\mathbf{v}$ is an externally imposed convection velocity, and b is a parameter that limits the population number density to a maximum saturation value. If $\mathbf{v}=0$ and $U(\mathbf{x})$ is constant, Eq. (1) corresponds to the Fisher wave equation [10] which has a solution with a limiting constant value of the front speed v_F. Wakita *et al.* [11] have studied a colony of *Bacillus subtilis* in a high nutrient and low agar medium growing in such a Fisher mode.

The two new features of the forced convection model given by Eq. (1) are the introduction of a growth potential $U(\mathbf{x})$, corresponding to exposing photosensitive bacteria to a light source, for example, and the convection of the bacteria due to the motion $\mathbf{v}$ of the light source. By considering a colony confined to a rectangular region, the resulting steady-state number density of the bacteria [the time-independent solution of Eq. (1)] was obtained in Ref. [9] as a function of $\mathbf{v}$. They concluded that the total number of bacteria in the rectangular region decreases linearly to zero as v approches v_F from below. The steady-state spatial density distribution was obtained by solving for the time-independent solutions of Eq. (1) numerically. Because the linearized version of Eq. (1) allows a mapping to non-Hermitian quantum mechanics, additional predictions of the properties of bacterial colonies in terms of localization-delocalization transitions in quantum systems can be made [8].

We report an experimental study of a *Bacillus subtilis* colony forced to migrate by environmental changes due to a moving ultraviolet (UV) source. UV radiation is shined on a Petri dish containing nutrient-rich agar except in a rectangular region which is shielded. Although UV radiation is supposed to kill these bacteria [12], we find more subtle behaviors. For example, the colony is confined to the shielded region only when the temperature is below a ''crossover'' value of approximately 22 °C. When the UV radiation is turned off, the front of the colony, which was near the boundary between the hostile and favorable regions, initially grows slowly, but recovers the Fisher front speed v_F in about 25 h. This slow recovery near the boundary suggests the presence of signaling between the bacteria, a feature which is absent in Eq. (1).

To study the effect of a changing environment, we rotate the rectangular shield with a constant angular velocity relative to the Petri dish. The bacteria are inoculated along a line inside the rectangular shield region. The rotation results in the colony being forced to convect with velocities that increase linearly from zero at the axis of rotation to a maximum value at the edge of the plate. We find that the bacteria colony cannot keep up with the shielded region if the shield moves with velocities much greater than v_F, thus showing an extinction transition in qualitative agreement with the theoretical model [9]. The spatial number density $n(\mathbf{x},v)$ of the bacteria as a function of the speed v was measured and found to be time dependent, even after 3 days of forced convection. These experimental results illustrate the rel-

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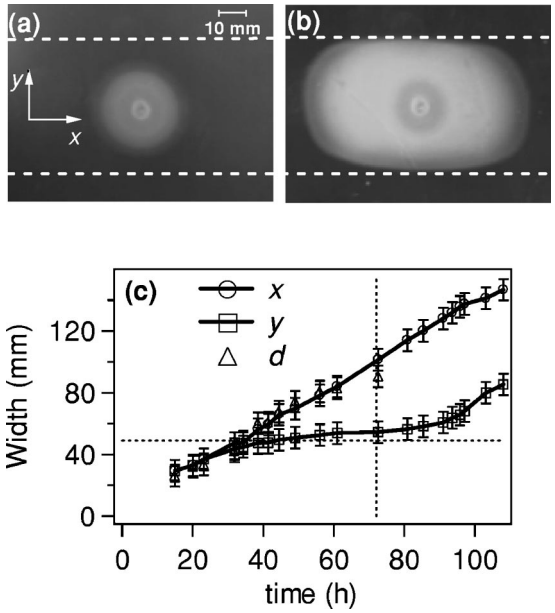

FIG. 1. (a) An image of a *Bacillus subtilis* colony growing on nutrient-rich agar at $t=23.15$ h after point inoculation. The region inside the dashed line is shielded from UV radiation. (b) At a later time ($t=65.45$ h), the circular front is distorted due to the confinement of the bacteria to the shielded region. (c) The width of the bacterial colony along the shield (x) and perpendicular to the shield (y). The time dependence of the diameter d of a colony growing in a Petri dish which is completely shielded from UV radiation is also shown. The horizontal dotted line corresponds to the boundary of the shielded region, and the vertical dotted line corresponds to the time when UV radiation is switched off. The temperature in all cases was $21\pm0.5\,^{\circ}$C.

evance of Eq. (1) and also indicate that the time-dependent response is of experimental relevance because of the long time scales of biological systems.

We now describe our experimental procedure and observations in more detail. The wild-type strain of *Bacillus subtilis* was obtained from Presque Isle Cultures and freeze dried at $-70\,^{\circ}$C. All experiments were performed from this initial sample by incubating a portion of the sample for 8 h at $30\,^{\circ}$C in nutrient-rich broth. A drop of this broth representing a total of at least 10^7 bacteria is used to inoculate the nutrient-rich agar. The experiments were performed in 15-cm-diam Plexiglas Petri dishes containing a thin layer of nutrient agar (7 g/l of bacto-peptone and 3 g/l agar.) These conditions are similar to that used in previous observations of the Fisher wave mode [11]. When inoculated as a single point source (diameter ~3 mm), the growth of the colony was observed to have a uniform disk shape with a front velocity that increases slowly for the first 8 h and eventually reaches a constant front speed v_F consistent with previous work [11]. Experiments were performed over a range of temperature ($21-40\,^{\circ}$C) and it was found that v_F is an increasing function of temperature within this range with $v_F=1.7$ μm/s at $40\,^{\circ}$C and $v_F=0.19$ μm/s at $21\,^{\circ}$C.

Next we describe our experiments in which we shine UV radiation on the Petri dish using two 8-W long-wavelength UV lamps placed 5 cm above the dish. An aluminum sheet is used to shield a rectangular region of the Petri dish from the radiation (see Fig. 1). The density of the bacteria is obtained by imaging the light scattered by the bacteria with a charge coupled device (CCD) camera. Calibration experiments show that the light intensity is proportional to the bacteria density. The colony at time $t=23.15$ h after a point inoculation at the center of the Petri dish is shown in Fig. 1(a) for $21\,^{\circ}$C. The shielded region is within the dashed lines and has a width $w=5$ cm. We observe that the front of the colony is circular and its diameter is smaller than w. As the colony grows farther outward, the edge of the shielded region is reached, and the shape of the colony is no longer circular as shown in Fig. 1(b). The width of the colony along the axes parallel (x) and perpendicular (y) to the shield is plotted in Fig. 1(c). The error bars correspond to the range of fluctuations due to slightly different initial conditions in different runs. The diameter d of the bacterial colony growing at the same temperature in a Petri dish which is completely shielded from UV radiation is also shown. We observe that the colony under the shield grows with a speed comparable to v_F at that temperature. As the colony approaches the edge of the shield, the front speed slows down because the bacteria are confined.

To further demonstrate that the confinement effect is due to the presence of UV radiation, the UV radiation was turned off after 72 h. We observe no change in the velocity of the front along the x direction as expected, because the bacteria are deep inside the shield. However, we would expect to see a change in the rate of growth along the y direction because the radiation has been removed. We observe that the front velocity recovers to v_F, but only after 25 h. This behavior is not modeled by Eq. (1), but is important in our discussion of the convection experiments as discussed below.

We performed experiments at higher temperatures and observed that for temperatures greater than approximately $22\,^{\circ}$C, the bacteria are able to grow into irradiated regions, but with a front speed that decreases with time. (At $26\,^{\circ}$C, the speed was reduced by 41% after 12 h.) Hence, in the presence of radiation we can vary the growth rate by changing the temperature and obtain a transition from a localized colony to one which is delocalized. A detailed study of this phenomena would be an interesting avenue for further research. In this paper we will consider a simple case in which the bacteria are confined at a temperature of $22\pm1\,^{\circ}$C to investigate the extinction transition in the presence of convection.

The convection experiments were performed by inoculating the bacteria along a diameter of the Petri dish. The Petri dish is then kept under a radiation shield of width $w=4.3$ cm and placed on a rotating platform, similar to the experiments described earlier. As the platform rotates, the region shielded from the UV radiation advances at a speed which increases linearly from the axis of rotation outward. The colony was initially allowed to grow for 14 h before the platform was rotated. During this time the bacteria covered the shielded region. The time $t=0$ corresponds to the time at which the platform was rotated. The results of the colony growth under these conditions are shown in Fig. 2. The position of the shielded region and the axis of rotation are indicated. The bacterial population is clearly seen to migrate and follow the shielded region at low velocities near the axis of rotation and lag behind at higher velocities.

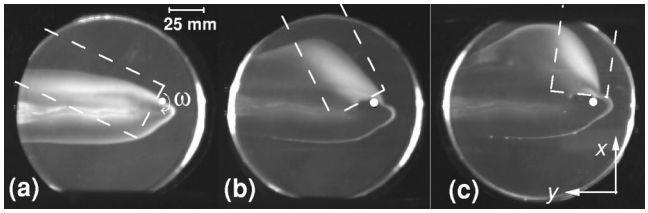

FIG. 2. Images of the bacterial colony when the shielded region is rotated at a constant angular velocity $\omega = 6.69 \times 10^{-6}$ s^{-1}. (a) $t = 24$ h, (b) $t = 46.56$ h, and (c) $t = 73.73$ h. The imposed convection velocity increases linearly from the axis of rotation. The bacteria follow the shielded region at low values of v, but lag behind far from the axis of rotation corresponding to higher velocities of the shield.

These observations are consistent with the theory of Ref. [9] where a phase diagram for the growth and the extinction of a colony as a function of the growth potential U and the convection speed v was obtained using Eq. (1). In particular, it was predicted that the bacteria will be localized at the favorable region. Furthermore, the total bacterial population in favorable regions decreases linearly to zero as a function of v as v approaches v_F from below. (In this theory the critical extinction speed v_c is the same as v_F.) To make quantitative comparisons, we have extracted the positions of the fronts corresponding to the three images shown in Fig. 2. These positions are plotted in Fig. 3(a); the origin corresponds to the axis of rotation and the initial line of inoculation is along the horizontal axis. The dashed arc in Fig. 3(a) corresponds to the distance where the velocity of the shield is the same as v_F. We observe that very far from the axis of rotation, the front does not change during the time $t = 46.56$ h to $t = 73.73$ h, indicating that bacteria which could not cope

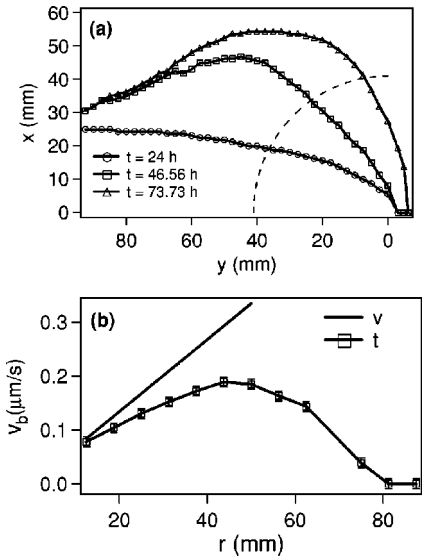

FIG. 3. (a) The colony fronts extracted from the images shown in Fig. 2. The axis of rotation is at (0,0) and the initial line of inoculation is along the horizontal axis. The distance where the speed of the shield corresponds to the Fisher wave velocity of the bacteria around 22 ° is shown by the dashed circle. (b) The speed of the bacteria front v_b is observed to increase to 0.23 μm/s and then decrease. This maximum speed corresponds to $v_F = 0.26$ μm/s around 22 °C.

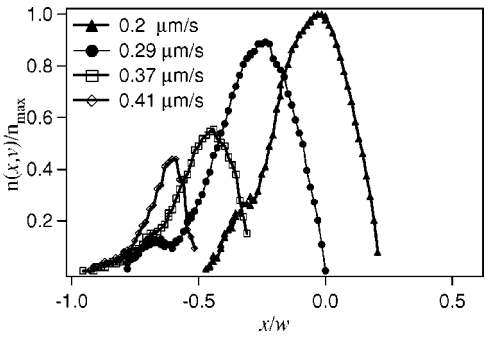

FIG. 4. The number density $n(x,v)$ of bacteria at various speeds v at $t = 73.73$ h normalized by the maximum number density $n_{\max}$. The horizontal axis is normalized with the width w of the shielded region and corresponds to 0.5 to -0.5. The density of the population is observed to decay to zero for higher velocities but the distribution is time dependent.

with the speed of the shield were left behind in the hostile irradiated region and did not grow.

Dividing the displacement of the bacteria front by the time difference between images, we extracted the approximate velocity of the front as a function of the radial distance r. Such an analysis ignores the diffusion of the bacteria along the radial direction. The data for the average velocity of the front, $v_b(r)$, are plotted in Fig. 3(b). The velocity of the shield $v(r)$ also is plotted to provide a reference for the front velocities. The bacteria are confined to the shielded region, and $v_b(r)$ is observed to increase with r, but is always less than $v(r)$, the corresponding speed of the shield. The reason for this lag might be due to the slow recovery of the bacteria after the UV-irradiated region moves ahead as discussed earlier in reference to Fig. 1(c). We also observe that $v_b(r)$ increases linearly up to a velocity of 0.2 μm/s which corresponds to $r \sim 45$ mm. For greater r, $v_b(r)$ decreases and the bacteria increasingly lag behind the shield and stop growing for $r > 80$ mm. The maximum value of v_b corresponds to the value of v_F of the bacteria colony at 22 °C in the absence of convection and UV radiation.

To explain the velocity data for $r > 50$ mm, we note the following. In the interval of time corresponding to the images shown in Figs. 2(b) and 2(c), the point where the bacteria completely lag behind the shield decreases from $r = 75$ mm to $r = 59$ mm. During this time, the bacteria are exposed to UV radiation for at least part of this time interval which increases for larger r. Hence, because the bacteria grow for only a portion of the total time, the mean front speed $v_b(r)$ decreases. The front speed is zero when the bacteria are always in the UV radiation corresponding to $r > 80$ mm in Fig. 3(b).

We also note that because of the slow rate of growth of the colony, the relative slow speed of the shield v, and the finite width of the shield w, a long transient time of the order of $w/(v - v_c)$ is required for the shield to leave the colony which is growing with a speed v less than the critical extinction speed v_c. This transient time diverges as v approaches v_c. Hence, for an experiment which is conducted over a finite duration, the value of r where the bacteria completely lag behind the shield is larger than the value corresponding to the critical extinction speed v_c. However, v_c can be indirectly calculated from the above relation for the transient

time. We obtain the estimate $v_c \sim 0.23$ μm/s which is similar to the value of $v_F \sim 0.26$ at 22 °C. This estimate was obtained from the image in Fig. 3 using $v = 0.4$ μm/s at $r = 59$ mm where the bacteria have completely lagged behind the shield at time $t = 73.73$ h of rotation. Therefore, we find a critical extinction speed consistent with the Fisher wave velocity as predicted in Ref. [9].

A more direct comparison of our experimental results to theory can perhaps be made by considering the time-dependent response of the model considered in Eq. (1). Additional considerations such as the time-dependent response of the front speed of the bacteria may have to be incorporated. To encourage future comparisons of experimental data with time-dependent models, we plot the number density $n(x,v)$ of the bacteria colony at different distances from the shield in Fig. 4 corresponding to different convection velocities v. The shielded region normalized by the width w corresponds to -0.5–0.5. These data correspond to the image shown in Fig. 2(c). These density distributions are still time dependent except at $v = 0.41$ μm/s, which corresponds to distances where the bacteria are immobile because they have been in the UV-irradiated regions for a long time. We observe that the front of the colony in the direction of the convection velocity always lags behind the edge of the strip. This characteristic of the bacteria distribution is similar to that predicted in Ref. [9], but a direct comparison is not possible because the distribution is still time dependent after $t = 73$ h of rotation. From Fig. 4 we further observe that the total bacterial population given by the area under the curve decreases for increasing velocity. We have found it impractical to conduct the experiments for a longer time, which is a significant limitation in making a more direct comparison with time-independent predictions.

The fact that the extinction transition occurs near v_F is an interesting result for real biological systems because of the relatively simple model considered in Refs. [8,9]. Our experiments are an important first step in investigating the usefulness of convection-diffusion models in studying convection in biological systems. The question remains whether the observed evolution of the front can be captured by the time dependence in Eq. (1) with the same initial conditions or whether additional terms which include the time-dependent interactions between the bacteria and the hostile environment are necessary.

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