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Localization and Extinction of Bacterial Populations under Inhomogeneous Growth Conditions

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ABSTRACT The transition from localized to systemic spreading of bacteria, viruses, and other agents is a fundamental problem that spans medicine, ecology, biology, and agriculture science. We have conducted experiments and simulations in a simple one-dimensional system to determine the spreading of bacterial populations that occurs for an inhomogeneous environment under the influence of external convection. Our system consists of a long channel with growth inhibited by uniform ultraviolet (UV) illumination except in a small "oasis", which is shielded from the UV light. To mimic blood flow or other flow past a localized infection, the oasis is moved with a constant velocity through the UV-illuminated "desert". The experiments are modeled with a convective reaction-diffusion equation. In both the experiment and model, localized or extinct populations are found to develop, depending on conditions, from an initially localized population. The model also yields states where the population grows everywhere. Further, the model reveals that the transitions between localized, extended, and extinct states are continuous and nonhysteretic. However, it does not capture the oscillations of the localized population that are observed in the experiment.

INTRODUCTION

The growth, spreading, and extinction of a population in an inhomogeneous environment are of interest given the global decline in biodiversity. Under what conditions does a population change from one that is localized to one that spreads throughout the available domain? This question is important not only for species in the earth's ecosystem but also for understanding the transition from a localized to a systemic infection in an organism. Simple model systems can provide insight into the kinds of phenomena that can occur in these complex systems. Population growth studies often examine bacteria because of the short doubling time. Studies of bacterial growth in homogeneous environments have revealed the spontaneous formation of different spatial patterns in response to environmental stresses (Shapiro and Dworkin, 1997; Berg and Purcell, 1977; Ben-Jacob et al., 1994; Matsushita et al., 1998). We are interested in the situation more commonly found in nature: inhomogeneous environments.

Our work is motivated by recent theoretical studies of the Fisher equation, which was generalized to model growth in an inhomogeneous environment under the influence of a convective flow. Those studies indicated a transition from localized populations to delocalized populations that grow everywhere (Nelson and Shnerb, 1998; Dahmen et al., 2000; Shnerb, 2001). Experiments have also been conducted on growth in inhomogeneous environments, but the conditions were too complex to compare with the model studies (Neicu et al., 2000; Shnerb, 2001).

We have developed an experimental system for studying bacterial growth in an inhomogeneous environment. *Escherichia coli* bacteria grow in a long, thin channel with different growth conditions in a small section of it. The position of this different growth region changes in time. We measure the bacterial concentration as a function of space and time in experiments that typically last one week, which is long compared to the half-hour doubling time for the bacteria. The quasi one-dimensional geometry permits comparison with simple one-dimensional models.

The spreading of bacterial colonies measured for certain homogeneous conditions (Shapiro and Dworkin, 1997; Matsushita et al., 1998) in a petri dish has been found to be described by the Fisher equation (Fisher, 1937; Murray, 1989), which has traveling front solutions that propagate with a velocity $v_F = 2(Da)^{1/2}$, where a and D are, respectively, the growth rate and diffusion coefficient of the bacteria (Shapiro and Dworkin, 1997; Berg and Purcell, 1977; Ben-Jacob et al., 1994). Nelson and co-workers (Nelson and Shnerb, 1998; Dahmen et al., 2000) proposed a generalization of the Fisher equation to describe the growth of bacteria in an environment with a localized region of favorable growth conditions (an ''oasis'') that moves with a constant velocity v:

$$\frac{\partial c(x,t)}{\partial t} = D\nabla^2 c(x,t) + U(x - vt)c(x,t) - bc(x,t)^2. \quad (1)$$

The growth rate in the oasis ($|x| \le W/2$) is $U(x - vt) = a$, and in the surrounding ''desert'' ($|x| > W/2$) the growth rate is $U(x - vt) = -\epsilon a$, where W is the width of the oasis in a system of length L, as shown in Fig. 1 *a*. The average growth rate over the full length of the system is

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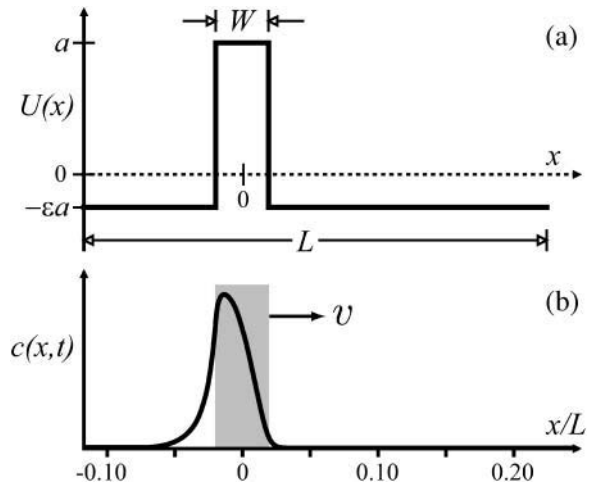

FIGURE 1 (*a*) Growth profile for a system with an oasis (width W and growth rate a) that moves with velocity v through a desert (width $L - W$ and growth rate $-\epsilon a$). (*b*) Example of a localized colony after 69 h of growth, from a numerical simulation of Eq. 1. The colony density is shown by the solid black line and the shaded band depicts the oasis. Simulation parameter values: $v/v_F = 0.9$, $a = 4.76 \times 10^{-4}$ s^{-1}, $D = 6 \times 10^{-5}$ cm^2/s, $\epsilon = 0.7$, $W = 2$ cm, $L = 50$ cm, and $b = 7.93 \times 10^{-11}$ cm/s. Concentration $c(x, t)$ is in arbitrary units.

$$\frac{\langle U(x - vt)\rangle}{a} = \frac{W - \epsilon(L - W)}{L}, \tag{2}$$

which we call the average terrain. An analysis of a linearization of Eq. 1 by Nelson and co-workers (Nelson and Shnerb, 1998; Dahmen et al., 2000) suggested that qualitatively different spatial distributions of concentration would occur for different values of the oasis velocity v and the average terrain (Eq. 2). Numerical simulations (Dahmen et al., 2000) of the full nonlinear equation suggested that these qualitative changes occur not over a range of parameter values, but rather at particular thresholds of these two parameters; however, this was not investigated in detail.

Extinction, localization, and delocalization

For oasis velocities v exceeding a critical value v_c and sufficiently harsh terrains (i.e., for $\langle U\rangle/a$ sufficiently negative), life is not sustainable and the population becomes extinct, approaching zero concentration everywhere.

For $|v| < v_c$, there is a range of values of the terrain $\langle U\rangle/a$ for which the population remains localized, growing in the oasis while decaying exponentially with distance from the oasis. An example of such a localized state is pictured in Fig. 1 *b*. The population is sustained in the moving oasis but dies out in the desert.

Finally, for a sufficiently rich terrain, the population grows everywhere, for any value of the oasis velocity v. No matter how the population is initially distributed throughout the length of the system, the population becomes delocalized.

The identification of the distinct sustainable localized, delocalized, and extinct states, and the suggestion that there could be well-defined transitions among them, originally resulted from an analysis of a linearized version of Eq. 1, which disregards the biologically important saturation term (Nelson and Shnerb, 1998; Dahmen et al., 2000). Transitions between the different regimes can be understood only by including the nonlinear saturation term. We have performed numerical simulations of the full nonlinear equation to compare with the laboratory observations and to study the transitions among the localized, delocalized, and extinct states.

METHODS

We examined bacterial growth in a rectangular channel of length $L = 25$ cm, thickness 0.2 cm, and height 2.5 cm. This thin quasi one-dimensional geometry was chosen so that the observations could be compared to predictions of one-dimensional models (Dahmen et al., 2000). Most of the 25 cm long channel was exposed to ultraviolet (UV) light to inhibit bacterial growth, but inside this ''desert'' was a short ($W = 3$ cm) ''oasis'' where the UV light was blocked by a mask (see Fig. 1 *a*).

The species selected for this study was *E. coli* RW 120 because, for these bacteria, UV exposure results in cell death rather than mutation (the two mechanisms for repair of DNA are switched off) (Ho et al., 1993). Thus, as in the model, the population dies out in the desert if it is sufficiently harsh.

The rectangular channel was filled before each experimental run with a define media in a 0.2% agar gel maintained at 37°C; this nutrient level supported steady bacterial growth for at least 1 week. The purpose of the gel was to inhibit uncontrolled convection while allowing diffusion of the bacteria. A 120 cm long mercury lamp positioned 8.5 cm above the cell provided 10 W/m^2 UV illumination for the region not shielded by the mask.

Each run was initiated by dragging a needle that had been dipped into bacteria across the gel surface underneath the mask, thus infecting only the oasis region. We waited several hours for the bacteria to reach a saturated concentration $c_s = a/b$ in the oasis before starting to move the oasis with a constant velocity v, as in Fig. 1 *b*. Runs were made for oasis velocities ranging from 1.5×10^{-5} cm/s, where the oasis moved only 13 cm in an experiment lasting 10 days, to 3.6×10^{-4} cm/s, where the oasis traversed the 25 cm cell length in 19 h. At high oasis velocities, the original population could not keep up with the moving oasis, and the population decreased and became extinct; we continued to monitor the decline in the original population for several days after the oasis reached the end of the cell. During a weeklong experiment, most of the live bacteria remained close to the (oxygenated) air-gel interface, whereas dead bacteria sunk to the bottom of the channel in ~2 days.

The bacterial concentration near the gel surface was determined by measuring the transmission of light through the thin dimension of the cell. The light source was a 4.5 mW diode laser with a 670 nm wavelength and a 1 mm beam diameter. The light source and a detector were mounted on a translation stage with the center of the beam passing through the gel 1 mm below its surface. Each spatial scan of the bacterial concentration took 7 min, a time short compared to the time for the concentration to change significantly; thus the measurements yielded $c(x, t)$ at all positions x and times t for the duration of an experiment (3–10 days).

Experimental determination of the model parameters

We have measured the parameters in the model: the growth rate a of *E. coli* in 0.2% agar define media, the growth attenuation coefficient ϵ of the UV light, the diffusion constant D, and the saturation constant b.

The growth rate of RW 120 was determined using a standard dilution plating technique (Runyen-Janecky, 2001, private communication) in which

the number of bacteria per unit volume was counted every half hour. The measurements, shown in Fig. 2, yielded $a = 4 \times 10^{-4}\ s^{-1}$.

To determine ϵ, we measured the bacterial death rate caused by the UV light and found $\epsilon = 28$; hence $\langle U \rangle / a = -24.5$. We chose this strong illumination intensity for a first exploration of the experimental phenomena to have good signal/noise. Future studies should develop instrumentation with improved signal/noise to study the interesting behavior of the model for an average terrain near zero. However, even with improved signal/noise, it will not be possible in practice to make extensive explorations of the parameters because of the long timescales of the phenomena.

The diffusion constant D was determined from measurement of the radially averaged front velocity v_F of a colony growing in 0.2% agar under homogeneous conditions in a petri dish, assuming that $D = v_F^2/2a$ (Murray, 1993; van Saarloos, 1987, 1988). For bacteria growing in a petri dish with 0.2% agar in define media, we measured $v_F = 2.4 \times 10^{-4}$ cm/s. This result together with the measured value of a yields $D = 7 \times 10^{-5}\ cm^2/s$.

A steady-state concentration c_s was achieved with point inoculation of bacteria in the oasis and no incubation period for $v/v_F \ll 1$. A value for the saturation parameter b, 7.93×10^{-11} cm/s, was determined from the relationship $b = a/c_s$.

We also conducted some measurements in 0.1% agar Luria Broth media where a and D were larger. These measurements yielded $a = 6 \times 10^{-4}\ s^{-1}$, $v_F = 4.4 \times 10^{-4}$ cm/s, and $D = 1.6 \times 10^{-4}\ cm^2/s$.

Simulation

We numerically integrated Eq. 1 using the parameter values given in the previous section, except for ϵ, which was scanned over a range of values in the simulations (see Fig. 5). The value of v_F used in the simulations was typically $v_F = 3.38 \times 10^{-4}$ cm/s, the average of the values measured in the two different growth media. In the simulation, space was discretized using the method described in Dahmen et al. (2000) and Franosch and Nelson (2000). The resulting N-coupled ordinary differential equations (where the number of grid points N was typically 2032) were integrated in time from an initial condition of a Gaussian concentration profile with 10% noise centered in the favorable growth region (Mann, 2001). An alternative initial condition, a spatially uniform concentration with the saturation value a/b, also with 10% noise, yielded the same behavior at long times. Both periodic and reflective boundary conditions were found to yield extinct and localized solutions, whereas delocalized solutions were found only with periodic boundary conditions.

OBSERVATION OF LOCALIZATION AND EXTINCTION

Comparison of experiment and simulation

In the experiment, we varied a single parameter, the velocity v of the oasis. The observations are compared with the numerical simulations in Fig. 3: for large v, the population becomes extinct (*left column*) and for small v a sustained localized population develops (*right column*); the figure also shows an example of an intermediate value of v, where the population slowly becomes extinct (*middle column*). The model captures the observed qualitative dependence on v.

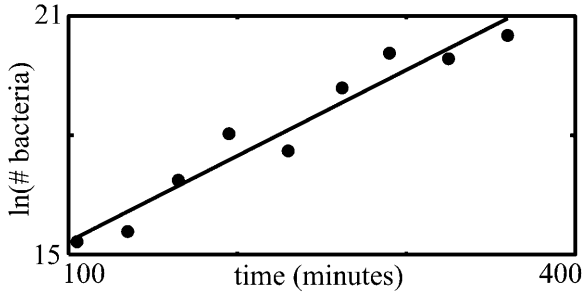

FIGURE 2 Experimental determination of the growth rate a (''#bacteria'' is the number of bacteria per 100 μml).

In the remainder of this subsection, we describe further the experimental observations, and in the next subsection we present results from the simulations, where an extensive exploration of the control parameter space was possible, including the parameter range yielding delocalized populations, which were not studied in the experiments because of the long evolution times required and because the current setup has nonperiodic boundary conditions.

In the case shown in Fig. 3 *a*, the bacteria inoculated in the oasis were first allowed to grow for 13 h with the oasis at rest before it began to move at 3.6×10^{-4} cm/s. The bacteria did not diffuse and grow fast enough to maintain a population within the moving oasis, and the original bacterial colony that was left behind became fully exposed to UV light. The bacteria then died and slowly sunk to the bottom of the channel, out of the field of the measurement of concentration; at 73 h some (presumably dead) bacteria were still present, but the population was declining toward zero. The waviness in the concentration profile is due to the inhomogeneous inoculation of the gel (see $t = 3$ h).

The development of stable localized populations in a moving oasis is illustrated in the column on the right of Fig. 3. The bacterial colony moves in the same direction as the oasis motion, and at least part of the colony remains in the oasis; bacteria that are left behind in the desert eventually die.

The spatiotemporal behavior of the colony under conditions close to the extinction-localization transition is shown in the middle column of Fig. 3. Close to this transition we observe in the experiment a double-peaked oscillating front, as can be seen in Fig. 4. These oscillations persist for many generations. The parameters in the simulations of the model Eq. 1 have been varied widely, but no oscillations have been found.

Transitions in bacterial populations

The 1 week time required for measurements for each set of parameter values made detailed laboratory investigations of the transitions impractical. However, the numerical simulations of Eq. 1 were conducted for wide ranges in $\langle U \rangle / a$ and v/v_F. The simulations revealed not only the three distinct states but also information on the transitions among these states: extinction-localization, extinction-delocalization, and localization-delocalization, as shown in Fig. 5. The phase diagram is plotted in parameter ranges near the transitions, whereas the experiments were run at $\langle U \rangle / a = -24.5$, off the plot. Simulations conducted using experimental parameters show that the phase diagram for those values can be extrapolated from Fig. 5.

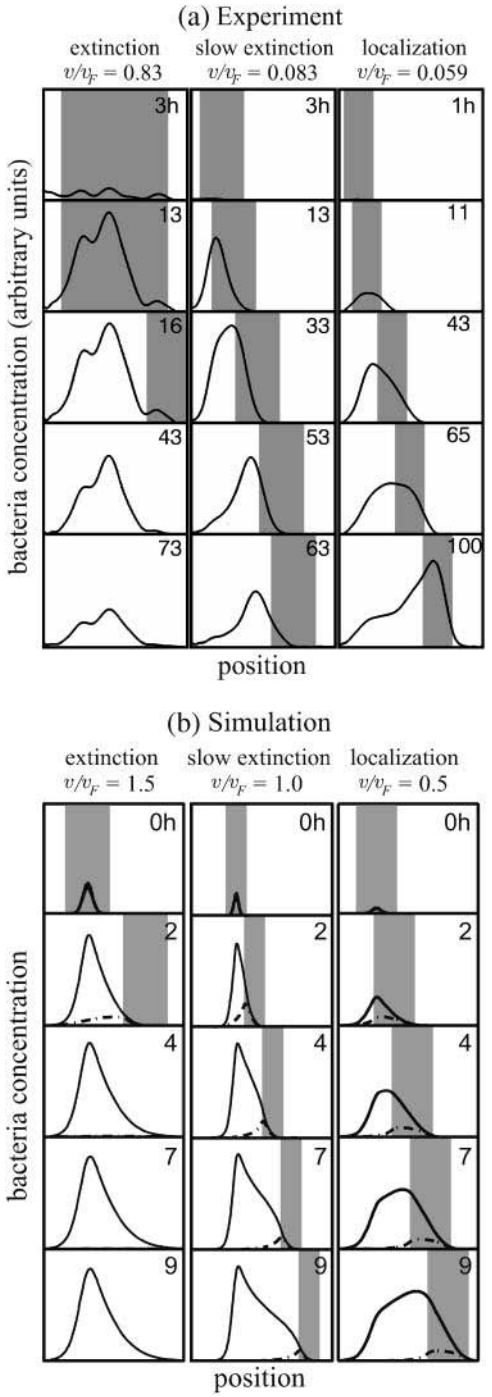

FIGURE 3 Bacterial colony evolution in (*a*) experiment and (*b*) numerical simulation of Eq. 1 for oasis velocities v leading to extinction (*left column*), slow extinction (*middle column*), and a sustained population in the oasis (*right column*). The shaded boxes show the location of the oasis (3 cm wide in a sample of length 25 cm); in the left column the oasis moves out of the region shown, leaving behind the population (which initially developed in a stationary oasis) exposed to the deadly UV radiation. The same thing happens in the middle column, but more slowly. The experimental measurements cannot distinguish between live and dead bacteria, but the results from the simulation show the concentration $c(x, t)$ of bacteria living at time t as dashed lines, whereas solid lines show the total concentration $\int c(x,t)dt$ of living and dead bacteria; thus at $t = 2$ h almost all of the bacteria are already dead in the simulation (*left column*). Other simulation parameters are given in Fig. 5. For the simulations, the bacterial concentration varies from 0 to 1.97 *a*/*b*, 2.74 *a*/*b*, and 10.73 *a*/*b* in the left, middle, and right columns, respectively.

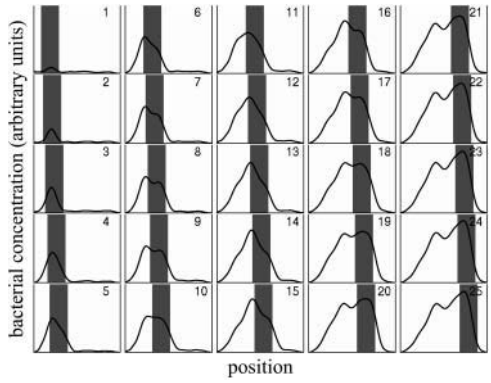

FIGURE 4 Snapshots taken hourly in the experiments reveal spatio-temporal oscillations of the bacterial concentration. The shaded boxes show the oasis position. The velocity of the oasis was $v = 1 \times 10^{-4}$ cm/s, and the experiment was conducted for a 0.1% agar gel with Luria Broth media.

Studies of behavior near the transitions were made with v/v_F changed in steps as small as 0.0001 and $\langle U \rangle / a$ changed in steps as small as 0.0005. These studies showed that each transition was continuous, as Fig. 6 illustrates. In addition, scans were made for both increasing and decreasing values of the control parameters, and the results were found to be independent of the direction in which the control parameter changed; this absence of hysteresis provides further evidence that the transitions are continuous.

To characterize the transitions, we examined the relaxation of the numerical solution to Eq. 1 after a change in control parameter values. In each case, the bacterial concentration was found to relax exponentially to its final value. Thus a characteristic decay time τ could be determined for each set of control parameters. These times became very long as a transition was approached, indicating the critical slowing down that is characteristic of supercritical bifurcations (Cross and Hohenberg, 1993). The divergence in relaxation time as the localization-extinction transition is approached is shown in the inset of Fig. 6 *a*.

For $\langle U \rangle / a$ large enough, the average terrain experienced by each bacterium was sufficient to support life everywhere; the population, initially limited to the oasis, became delocalized. However, the population remained larger in the oasis region, as Fig. 7 illustrates for a range of increasing oasis velocities.

DISCUSSION

A system's response to changes in local and global conditions is part of the knowledge required for applications such as achieving precision drug delivery, e.g., localizing a viral vector in its target cell during gene therapy. The

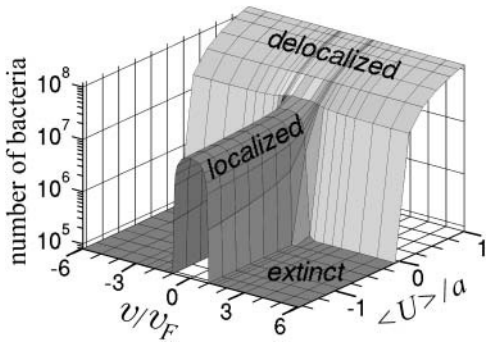

FIGURE 5 Phase diagram computed for the model, Eq. 1, showing the total number of bacteria in the system as a function of the two control parameters, the average terrain $\langle U \rangle / a$, and the oasis velocity v/v_F. There are three sustainable states: localized, where the bacterial colony survives only in the moving oasis; delocalized, where the bacterial population grows throughout the system, although the growth is largest inside the oasis region (see Fig. 7); and extinction, where the bacteria die out everywhere, even inside the moving oasis. Note that the diagram is symmetric about $v/v_F = 0$. The parameter values for the simulation were: $a = 4.76 \times 10^{-4}$ s^{-1}, $b = 7.93 \times 10^{-11}$ cm/s, $D = 6 \times 10^{-5}$ cm^2/s, $W = 3$ cm, $L = 20.32$ cm, and $v_F = 3.37 \times 10^{-4}$ cm/s.

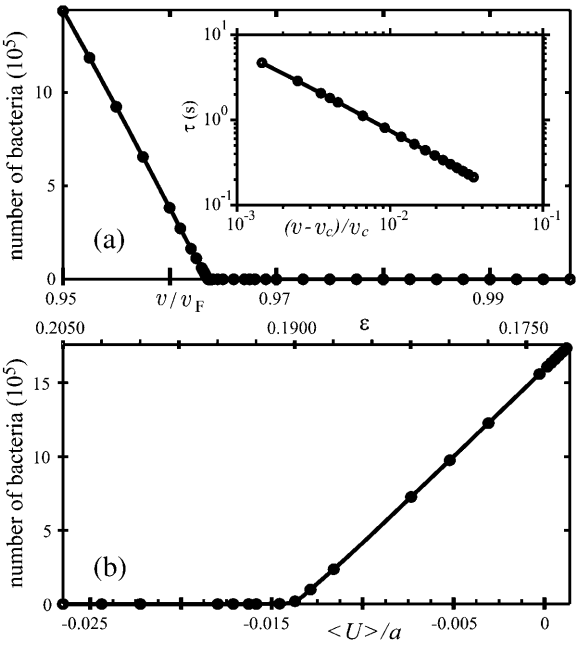

FIGURE 6 (*a*) Transition from a population localized in the oasis to extinction of all bacteria as the normalized oasis velocity v/v_F was increased in a simulation of Eq. 1; the average terrain was held fixed, $\langle U \rangle / a = -0.49$. The inset illustrates critical slowing down as the transition at $v = v_c$ was approached from above: the relaxation time diverges, $\tau \propto (v - v_c)^{-\gamma}$, with $\gamma = 0.98 \pm 0.03$. Similar results were obtained when approaching the transition from below. (*b*) The transition between extinction and delocalization with fixed $v/v_F = 2$, as a function of $\langle U \rangle / a$ (or, as shown along the *top* of the graph, as a function of the attenuation factor ϵ). Other parameter values are given in Fig. 5.

moving oasis could correspond to a flow of plasma past a fixed region of favorable growth conditions.

Our study has concerned bacterial populations that initially grew in a stationary oasis that lay within a desert; then the oasis was made to move with a constant velocity. We found that the population could be sustained or could die out, depending on the oasis velocity and the average terrain, which depended on harshness of the desert and the size of the oasis relative to the size of the desert. Results from our numerical simulations of Eq. 1 are in qualitative accord with the laboratory observations, although the model fails to capture the observed double-peaked oscillations found for the spatial distribution of bacteria in the moving oasis. An alternative model might include (in addition to diffusion, convection, and growth) the internal dynamics of various gene expression networks within individual bacteria. Such a model could produce oscillations as the protein levels change in response to environmental stresses. Temporal oscillations of various proteins within cells have been demonstrated in *E. coli* (Elowitz and Leibler, 2000). Similar temporal oscillations have been captured in a minimal model based on activator-repressor dynamics (Vilar et al., 2002).

The analysis of the linearized equations by Dahmen et al. (2000) revealed a transition between delocalized and mixed steady states for $v_c = 0.92\ v_F$, whereas our numerical analysis of the full nonlinear equations, conducted in the range of biologically relevant parameters, yielded only sustained delocalized states, i.e., nonzero concentration everywhere (see Mann, 2001).

In conclusion, our numerical simulations of the model yield deeper insights into the mechanisms governing the colony behavior than could be obtained from the experiments alone. In particular, the simulations showed that the transitions between the different states were continuous and nonhysteretic. Further, the simulations yielded a delocalized state, where the bacterial population grew throughout the entire system; this state was not accessible in the experiments.

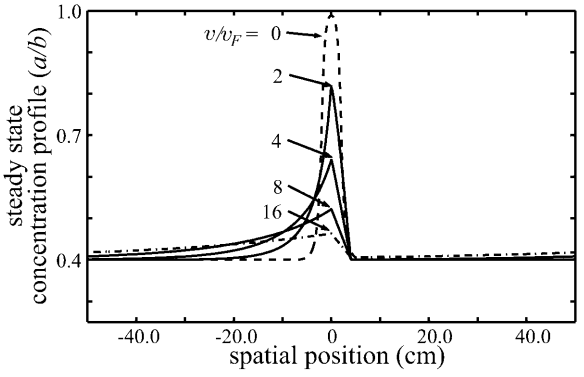

FIGURE 7 The asymptotic spatial distribution of an extended (delocalized) bacterial population computed in the reference frame of an oasis moving with different relative velocities v/v_F. The average terrain was held fixed, $\langle U \rangle / a = 0.424$, $D = 1.2 \times 10^{-4}$ cm^2/s, $v_F = 4.78 \times 10^{-4}$ cm/s, $a = 4.76 \times 10^{-4}$ s^{-1}, $\epsilon = -0.4$, $W = 4$ cm, $L = 1000$ cm, and $b = 7.93 \times 10^{-11}$ cm/s.

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