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Continuum model for the spatiotemporal growth of bacterial colonies

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A model for the spatiotemporal growth of bacterial colonies is introduced based on a reaction-diffusion equation with local and nonlocal modes of growth. If the local coarse-grained cell concentration is less than the physical close-packing concentration, local growth occurs. However, nonlocal growth occurs at the surface of a colony if local growth cannot account for the amount of growth required by the global growth law. The model is implemented for the radial growth of independent spherical colonies and applied to the growth of bacterial colonies immobilized within a gel.

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INTRODUCTION

The microbial growth kinetics of liquid cultures can be described in general by a nonautonomous differential equation of the form [1,2]

$$\frac{dN(t)}{dt}=\mu\alpha(t)N(t)F(N(t),N_m) \qquad (1)$$

with the initial value

$$N(t=0)=N_i \quad (N_i>0)\ , \qquad (2)$$

where $N(t)$ is the number of organisms in the system of volume V and μ is the growth rate. The term $\alpha(t)$ is an adjustment function which is included to account for any lag in the growth of cell number. This can result from inoculation with stationary phase cells or from a change in the environment for the inoculated cells. Thus $\alpha(t)$ can be regarded as a function switching on cell growth and it is assumed that $\alpha(t)$ is a monotonic increasing function of t with $\alpha(t=0)=0$ and $\alpha(t\rightarrow\infty)=1$. A convenient simple representation for $\alpha(t)$ is $\alpha(t)=\theta(t-t_L)$, where $\theta(x)$ is the usual Heaviside theta function [$\theta(x)=0$ if $x<0$ and $\theta(x)=1$ if $x>0$] and t_L is the lag time. The bulk growth function $F(N(t),N_m)$ sets the maximum cell number in the sample N_m and defines the specific form of the bulk growth law. For example, the generalized Verhulst equation for population growth is characterized by $F(N,N_m)=[1-(N/N_m)^{\nu}]/\nu$, which corresponds to logistic growth for $\nu=1$ and for $\nu=0$ reduces to the Gompertz equation [3].

The microbial growth curve of a batch culture is largely independent of whether the supporting growth medium is structured or unstructured, stirred or unstirred. Thus it is appealing to write a model for the spatiotemporal growth of bacterial colonies using a reaction-diffusion equation in which the reaction term is the bulk growth law applied at the microscopic level. However, this is not generally feasible, since the maximum cell concentration obtained in batch cultures is orders of magnitude smaller than the cell concentrations that can occur locally within a bacterial colony, which may be close packed. Thus a model for bacterial colony growth based upon a reaction-diffusion equation scheme must include two reaction terms to cope with growth that can occur locally, and growth that occurs nonlocally by reorganization and expansion of compact colonies through the division and growth of cells within the colony.

COLONY GROWTH

In unstirred media, since the growth in microbial number occurs by cell division, spatial variations of the microbial population can be expected and the spatiotemporal growth kinetics in the absence of fluctuations can be written as

$$\frac{\partial n(\mathbf{r},t)}{\partial t}=D\nabla^2 n(\mathbf{r},t)+\Lambda(\nabla n(\mathbf{r},t))^2 +\lambda n(\mathbf{r},t)G(n(\mathbf{r},t),n_m)\ , \qquad (3)$$

where D is the diffusion coefficient, ∇^2 is the Laplacian operator, and $n(\mathbf{r},\mathrm{t})$ is the local microbial number density with

$$N(t)=\int d\mathbf{r}\, n(\mathbf{r},t)\ , \qquad (4)$$

where the integral is taken over the sample volume. Since we are working within a continuum model framework the local number concentration has to be interpreted as a coarse-grained average of the number concentration over a local volume which is typically much greater than the cell volume. The third term on the right-hand side of Eq. (3) represents the local growth of cell number up to a maximum cell concentration of n_m characterized by a local growth rate λ and a local growth function $G(n,,n_m)$. A compact colony has a close-packed configuration of cells of concentration n_m corresponding to a volume fraction of $\phi_m=\pi n_m\sigma^3/6$, where σ is a length comparable with the mean cell diameter. Thus in general $n_m \gg (N_m/V)$. If the colony is unable to add cells locally due to packing constraints, growth must occur by the nonlocal addition of cells on the periphery as a result of the expansion and reorganization of the colony. This effect is accounted for by the second term on the right-hand side of Eq. (3), which corresponds to nonlocal growth occurring at concentration gradients,

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most notably at the surface of the compact microcolony. This nonlocal growth contribution is required in systems where the diffusion constant is sufficiently small in respect to the local growth rate that the colony cannot spread by diffusion alone. Such cases arise when the bacteria are immobilized by a gel network within the growth medium. Then cell division occurs, creating a compact localized colony of cells centered on the location of the inoculated cell.

For the radial growth of a spherically symmetric colony from an inoculum located at the origin, Eq. (3) reduces to

$$\frac{\partial n(r,t)}{\partial t} = \frac{D}{r^2}\frac{\partial}{\partial r}\left[r^2\frac{\partial n}{\partial r}\right] + \Lambda\left[\frac{\partial n}{\partial r}\right]^2 + \lambda n(r,t)G(n,n_m)\ . \tag{5}$$

If we define $u(r,t)=n(r,t)/n_m$, $t'=\lambda t$, and $x=(\lambda/D)^{1/2}r$, for suitable functional forms of G it is possible to rewrite Eq. (5) as

$$\frac{\partial u(x,t')}{\partial t'} = \frac{1}{x^2}\frac{\partial}{\partial x}\left[x^2\frac{\partial u}{\partial x}\right] + \Psi\left[\frac{\partial u}{\partial x}\right]^2 + u(x,t')G(u(x,t')) \tag{6}$$

with $\Psi=(n_m\Lambda/D)$.

By way of example, consider the case of local logistic growth with $G(u)=(1-u)$, so that $u(x,t')$ satisfies

$$\frac{\partial u}{\partial t'} = \frac{1}{x^2}\frac{\partial}{\partial x}\left[x^2\frac{\partial u}{\partial x}\right] + \Psi\left[\frac{\partial u}{\partial x}\right]^2 + u(1-u)\ . \tag{7}$$

First note that when $\Psi=0$, Eq. (7) reduces to Fisher's equation [4], which is known to possess solitary wave solutions with an asymptotic velocity of 2 [5]. Furthermore, note that there exists a nonlinear transformation of the Fisher equation which shows that solitary wave solutions are also obtained from a generalized Fisher equation [6] in which

$$\frac{\partial u}{\partial t'} = \frac{1}{x^2}\frac{\partial}{\partial x}\left[x^2\frac{\partial u}{\partial x}\right] + \frac{\psi}{u}\left[\frac{\partial u}{\partial x}\right]^2 + u(1-u)\ . \tag{8}$$

The important difference between this generalized Fisher equation and Eq. (7) lies in the coefficient of the square gradient term. Solutions of Eq. (8) require $\partial u/\partial x \rightarrow 0$ as $u(x,t)\rightarrow 0$ such that $(\partial u/\partial x)/u$ remains finite, but Eq. (7) has no such restriction on the density gradient. Thus solutions of Eq. (7) can display a clear colony boundary at which the cell density is zero while the density gradient is nonzero.

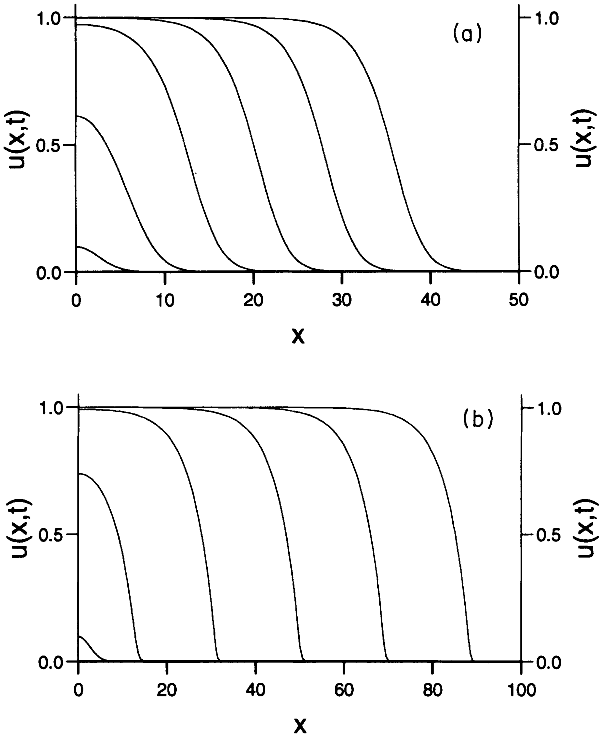

FIG. 1. Radial density profile $u(x,t')$ at $t'=0$, 4, 8, 12, 16, and 20 for the growth of a spherical colony from Eq. (6) with (a) $\Psi=1$ and (b) $\Psi=25$. Gaussian inoculation with $n(0,0)/n_m=0.1$ and $\omega=2.5(D/\lambda)^{1/2}$.

To investigate the structure of solutions of Eq. (7), consider the growth of a colony from an inoculum centered on the origin whose density profile is described by a Gaussian with

$$n(r,0)=n(0,0)\exp(-r^2/2\omega^2)\ , \tag{9}$$

where $n(0,0)$ and ω characterize the height and width of the colony at inoculation. Figure 1 shows the time evolution of the colony profile following inoculation for values of $\Psi=1$ and 25. For $\Psi=1$ the results for the colony profile are indistinguishable on the scale of the figure with those from the Fisher equation with $\Psi=0$ and for all $\Psi>0$ we find solitary-wave behavior after the initial transient period. As Ψ increases there is an increasing asymmetry of the wave front associated with an increase in the front velocity. The wave-front velocity is found to be a monotonic increasing function of Ψ, but for $\Psi>10$ the rate of increase of the front velocity with Ψ is slower than linear.

Ψ represents the key parameter controlling colony growth within this model and its relationship to the physical processes controlling colony growth is of some importance. In some cases it might be argued that the gradient contribution to the growth of the colony should be position dependent and proportional to the local number of growing cells, i.e., $\Psi \propto \partial n/\partial t$. (For regions of the colony where $n \ll n_m$ this corresponds to local exponential growth with $\Psi \propto n$.) Then we might write $\Psi=\psi u G(u)$ and Eq. (6) would become

$$\frac{\partial u}{\partial t'} = \frac{1}{x^2}\frac{\partial}{\partial x}\left[x^2\frac{\partial u}{\partial x}\right] + uG(u)\left[1+\psi\left[\frac{\partial u}{\partial x}\right]^2\right]\ . \tag{10}$$

For the case of local logistic growth with $G(u)=(1-u)$, this equation, like Eq. (7), displays solitary wave solutions with growth restricted to active regions near the periphery of the colony. But significantly in Eq. (10), diffusion represents the only mechanism for spreading the colony in space and thus in the limit of $D\rightarrow 0$, i.e., small bacterial diffusion constants, the colony would be unable to expand. Thus we expect the coefficient Λ to be proportional to the amount of total growth within the colony, i.e., $\Lambda \propto dN(t)/dt$, and not the amount of local growth.

GLOBAL CONSTRAINTS

The bulk growth laws which globally constrain colony growth are obtained in stirred, aerated, nutrient-rich growth media. Under the assumption that colony growth in unstirred media occurs under the same environmental conditions and is not limited by the diffusion of oxygen (for aerobic growth) or nutrient to the bacterial colony, it follows directly from Eqs. (3) and (4) that

$$\begin{aligned}\frac{dN}{dt} &= \int d\mathbf{r}\frac{\partial n}{\partial t}\\ &= \Lambda \int d\mathbf{r}(\nabla n(\mathbf{r},t))^2 + \lambda \int d\mathbf{r}\, n(\mathbf{r},t)G(n(\mathbf{r},t),n_m)\ ,\end{aligned} \tag{11}$$

Gauss's theorem ensuring that there is no contribution on the right-hand side of Eq. (11) from the diffusion term on the right-hand side of Eq. (3). When combined with Eq. (1) this gives

$$\begin{aligned}\mu\alpha(t)N(t)F(N,N_m) &= \Lambda \int d\mathbf{r}(\nabla n(\mathbf{r},t))^2\\ &\quad + \lambda \int d\mathbf{r}\, n(\mathbf{r},t)G(n(\mathbf{r},t),n_m)\ .\end{aligned} \tag{12}$$

The nonlocal and local growth coefficients Λ and λ may in principle be functions of position. But here, for simplicity, they will be assumed to be independent of position and may be determined at any time t for a given cell density distribution from Eq. (12) if we subject the system to constraints on the relative contributions from local and nonlocal growth. Here we shall apply the constraints that (i) the nonlocal growth contribution cannot be negative and (ii) growth occurs locally if possible with a local growth rate that cannot exceed the global growth rate. So it follows that if we require $\Lambda \geq 0$ then

$$\mu\alpha(t)N(t)F(N,N_m) \geq \lambda \int d\mathbf{r}\, n(\mathbf{r},t)G(n(\mathbf{r},t),n_m)\ . \tag{13}$$

If we further require $\lambda \leq \mu$ and growth to be local if possible, then

$$\lambda = \min\left[\mu, \frac{\mu\alpha(t)N(t)F(N,N_m)}{\int d\mathbf{r}\, n(\mathbf{r},t)G(n(\mathbf{r},t),n_m)}\right]\ . \tag{14}$$

Thus Λ and λ are determined by Eqs. (12) and (14). Note that if $\mu = \lambda$, then $\Lambda = 0$, whereas if $\lambda < \mu$ we have $\Lambda > 0$.

In the limit of $D \to \infty$, the cell density distribution will become uniform with $n(\mathbf{r},t) = N(t)/V \leq N_m/V \ll n_m$ for all $\mathbf{r}$. Thus in this limit we would have $G(n,n_m) \approx 1$ and so from Eq. (14) $\lambda \approx \mu\alpha(t)F(N,N_m)$. This corresponds to the mean-field limit, which is thus correctly included within the model.

Since $n_m \gg N_m/V$ and the inoculated cells can be assumed to be well dispersed throughout the system, growth can be well approximated by the evolution of a set of independent colonies. The radial growth of an independent spherically symmetric colony from an inoculum located at the origin is given by Eq. (5) with the global constraint

$$\mu\alpha(t)F(N) = 4\pi N_i \int dr\, r^2 \left[\Lambda \left(\frac{\partial n(r,t)}{\partial r}\right)^2 + \lambda n(r,t)G(n(r,t),n_m)\right]\ , \tag{15}$$

where N_i is the initial number of cells in the system. Equations (5) and (15) must be supplemented by the initial condition that $n(r,0) > 0$, which is met by assuming that the inoculation is in the form of a Gaussian centered on the origin as given in Eq. (9). For the case of a system with a local logistic growth law the equation for colony growth reduces to

$$\frac{\partial u}{\partial t^*} = \frac{1}{s^2}\frac{\partial}{\partial s}s^2\frac{\partial u}{\partial s} + \Psi\left(\frac{\partial u}{\partial s}\right)^2 + \frac{\lambda}{\mu}u(1-u) \tag{16}$$

with $s = (\mu/D)^{1/2}r$ and $t^* = \mu t$. If in addition the system is globally constrained by a global logistic growth law with

$$\frac{dU}{dt^*} = U(1-U)\ , \tag{17}$$

where $U(t) = N(t)/N_m$, then each independent spherical colony will grow according to Eq. (16) subject to the constraint that

$$U(1-U) = \zeta\left(\frac{N_i}{N_m}\right)\int ds\, s^2\left[\Psi\left(\frac{\partial u}{\partial s}\right)^2 + \frac{\lambda}{\mu}u(1-u)\right]\ , \tag{18}$$

where $\zeta = 4\pi n_m (D/\mu)^{3/2}$. Since we require $\Lambda \geq 0$, it follows that

$$U(1-U) \geq (\lambda/\mu)\zeta(N_i/N_m)\int ds\, s^2 u(1-u) \tag{19}$$

and so requiring growth to be local if possible with $\lambda \leq \mu$ gives

$$\frac{\lambda}{\mu} = \min\left[1, \frac{U(1-U)}{\frac{N_i}{N_m}\zeta\int ds\, s^2 u(1-u)}\right]\ . \tag{20}$$

Thus if $\lambda < \mu$ we have $\Psi = 0$, but if $\mu = \lambda$ then $\Psi > 0$ and is given by Eq. (18). The numerical results for the case of growth from a Gaussian inoculation according to Eq. (9) with $n(0,0) = n_m$ and $(\mu/D)^{1/2}\omega = [\zeta(\pi/2)^{1/2}]^{-1/3}$ to ensure that the initial colony corresponds to a single cell are given in Fig. 2 for $N_i/N_m=10^{-4}$ and $\zeta=1$ with fixed boundary conditions. This figure shows that for times at which $dN/dt>0$ the wave front propagates with a velocity that is not constant, a direct result of the time dependence of the nonlocal contribution to the colony growth arising from the constraints of the global growth law. For large times when $dN/dt=0$, and colony growth by the addition of new cells ceases, the colony spreads by diffusion. Decreasing N_i/N_m will lead to increases in the maximum colony size, while increasing ζ will decrease the maximum colony size.

For the radial growth of compact spherical colonies immobilized within a gel we must consider the limit $D\rightarrow 0$. The solution of Eqs. (5) and (15) in this limit is found, after an initial transient, to be identical to the result expected from dimensional analysis with a close-packed spherical colony of radius $R(t)$ given by

$$R(t)=(\sigma/2\phi_m^{1/3})[N(t)/N_i]^{1/3}\ . \tag{21}$$

Figure 3 shows the experimentally determined [7] colony radius (in μm) against time (in hours) for *Salmonella typhimurium* immobilized by a 10% (w/v) gelatin gel in a nutrient-rich broth at 20 °C, *p*H 7.0 and 3.5% (w/v) NaCl. The bulk growth law for *Salmonella typhimurium* experimentally determined [8] under the same environmental conditions is shown in the inset of Fig. 3 which plots $N(t)$, the concentration of viable cells (cm^{-3}) against time (h). If we take $N_i=N(t=0)$ and $N_m=N(t=126)$, then the experimentally determined growth curve is well represented by a logistic growth law with theta function lag, namely,

$$dN/dt=\theta(t-t_L)\mu N(1-N/N_m) \tag{22}$$

with $\mu=0.275$ h^{-1} and $t_L=17.085$ h. (This fitted curve is also shown in the inset of Fig. 3). Using this global growth law in Eq. (21) determines the radial growth of immobilized independent spherical colonies and provides a good fit to the experimental data with $(\sigma/2\phi_m^{1/3})=1.15$ μm. Since we can expect ϕ_m to take a value somewhere in the vicinity of the close-packing volume fraction of hard spheres, typically $\phi_m\approx 0.64$, this would correspond to an effective spherical cell of diameter $\sigma\approx 2$ μm for *Salmonella typhimurium*, which is comparable with the known dimensions of the bacterium.

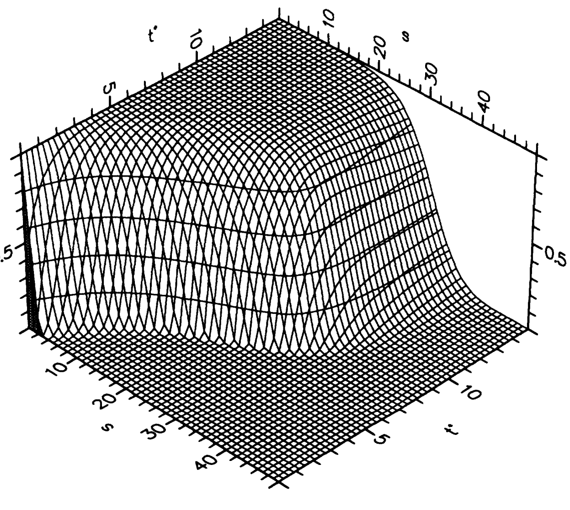

FIG. 2. Radial density profile $u(s,t^*)$ for the globally constrained growth of independent spherical colonies from Eqs. (16)–(18) with $\zeta=1$ and $N_i/N_m=10^{-4}$. Single-cell Gaussian inoculation with $n(0,0)=n_m$ and $(\mu/D)^{1/2}\omega=(\pi/2)^{-1/6}$.

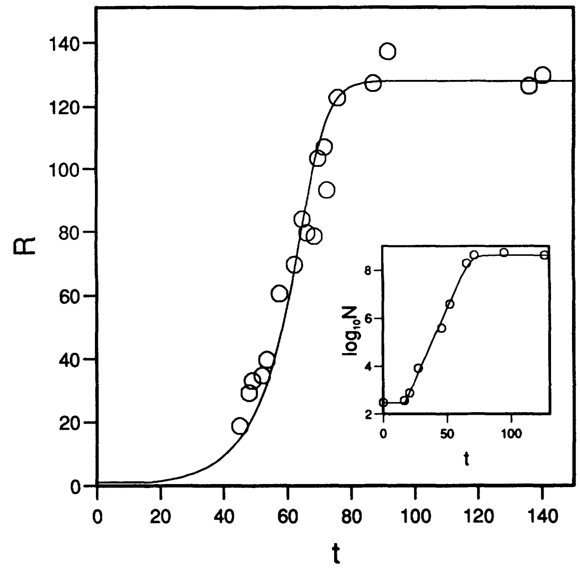

FIG. 3. Colony radius (μm) vs time (h) for *Salmonella typhimurium* immobilized by a 10% (w/v) gelative gel in a nutrient-rich broth at 20 °C, *p*H 7.0 and 3.5% (w/v) NaCl. Open circles denote experimental results, while the solid line shows the model result from Eqs. (21) and (22). The global growth curve for the same environmental conditions is shown in the inset, which plots the viable cell concentration (cm^{-3}) vs time (h).

DISCUSSION

In this paper a continuum model for the spatiotemporal growth of bacterial colonies has been introduced which utilizes the known macroscopic growth law as well as a reaction-diffusion equation for growth at the microscopic level. A key element of the model is the partition of the growth mechanism into two parts, one local and the other nonlocal. Nonlocal growth arises from the addition of cells at the surface of a compact colony as a result of colony reorganization following cell division within the compact colony.

The model presented here uses a single-component formalism for the growth of cell number within a colony. An important assumption is that the colony growth occurs under aerobic conditions in nutrient-rich growth media and is not limited by the diffusion of oxygen or nutrient to the colony. Thus the present formalism is not applicable to systems in which the colony growth is limited by the diffusion of material to or from the colony, as would be the case for the growth of bacterial colonies on the surface of a growth medium [9]. Extensions of this model formalism to include the coupled growth of cell biomass and cell number are possible. In addition, the explicit inclusion of concentration fields for nutrient and oxygen would also allow cases of diffusion-limited growth to be investigated.

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[1] B. D. Coleman, Math. Biosci. **45**, 159 (1978).
[2] R. R. Vance and E. A. Coddington, J. Math. Biol. **27**, 491 (1989).
[3] N. S. Goel, S. C. Maitra, and E. W. Montroll, Rev. Mod. Phys. **43**, 231 (1971).
[4] R. A. Fisher, Ann. Eugenics **7**, 355 (1937).
[5] S. Puri, K. R. Elder, and R. C. Desai, Phys. Lett. A **142**, 357 (1989).
[6] X. Y. Wang, Phys. Lett. A **131**, 277 (1988).
[7] A. R. Mackie (unpublished).
[8] T. F. Brocklehurst (unpublished).
[9] S. P. Fraleigh and H. R. Bungay, J. Gen. Microbiol. **132**, 2057 (1986).