AGGREGATION PATTERNS IN STRESSED BACTERIA

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We study the formation of spot patterns seen in a variety of bacterial species when the bacteria are subjected to oxidative stress due to hazardous byproducts of respiration. Our approach consists of coupling the cell density field to a chemoattractant concentration as well as to nutrient and waste fields. The latter serves as a triggering field for emission of chemoattractant. Important elements in the proposed model include the propagation of a front of motile bacteria radially outward from an initial site, a Turing instability of the uniformly dense state and a reduction of motility for cells sufficiently far behind the front. The wide variety of patterns seen in the experiments is explained as being due to the variation of the details of the initiation of the chemoattractant emission as well as the transition to a non-motile phase.

Over the past few years, there has been a significant increase in our understanding of how spatial patterns emerge via the propagating interfacial dynamics of nonequilibrium systems [1,2]. We have discovered how seemingly different systems can nonetheless exhibit strikingly similar behavior, due to the existence of nonequilibrium pattern selection principles. These principles rely on the idea that the final structure which emerges from an initially (linearly) unstable state is affected mostly by the nature of the instability, the possible existence of stable, highly nonequilibrium isolated steady-state structures (such as the single dendrite in nonequilibrium solidification), and the competition between globally ordererd arrangements of these structures as compared to more disordered morphologies. This framework has been applied to a variety of systems of physical, chemical and most recently [2] biological interest.

In this work [3], we analyze spot and stripe patterns seen in bacterial growth experiments; the first such results were due to Berg and Budrene [4] in e. coli. They found that cells could aggregate chemotactically, resulting in a wide variety of different colony structures ranging from arrays of spots to radially oriented stripes to arrangements of more complex elongated spots. In their study, e. coli were grown on single carbon source media and the appearance of patterns only in the case of highly oxidized nutrient suggested that respiratory byproducts (leading to oxidative stress [5]) trigger the observed chemotactic behavior. Two of their figures are reproduced in Fig.1. It is worth noting that this class of patterns is not limited to e. coli; similar structures have been seen in Salmonella typhimurium [7] and in Bacillus subtilis [6]. We suspect that this class of patterns is a universal “possibility” for microbial systems aggregating in the face of adversity.

Normally, bacteria divide and spread out into regions of initially low density. This can take the form of an expanding circle [9], or if some metabolic factor is in short supply, a (set of) expanding ring(s) [10]. In the presence of the aforementioned oxidative stress, the bacteria begin to emit a chemoattractant (believed to be asparagine) which causes them to aggregate via biasing their motion. Eventually, the bacteria turn non-motile, freezing the pattern into place. From the modeling point of view, the coupling of the chemoattractant diffusion equation to the bacterial density evolution leads to a Turing-like instability of the uniform density state. If we were to start the entire system at uniform bacterial density, patterns would develop as soon as the concentration of waste reaches a threshold value. These patterns would be defect-ridden, governed by the precise details of the initial conditions. This is indeed what was observed in the case of purposeful addition of hydrogen peroxide. The symmetric structures seen during growth from a single inoculated site are thus due to the interplay of the expansion with the Turing instability as triggered by the waste field, and possibly autocatalytically by the attractant field itself. Below, we will see how this occurs within our model.

Based on the above, we propose the following set of continuum equations for this system [5]:

\[ \dot{\rho} = D_\rho \nabla^2 \rho + G(\rho, u) - v_c \nabla \cdot (\rho \nabla c) - I[w] \rho \]
\[ \dot{\rho} = I[w] \rho \]
\[ \dot{n} = D_n \nabla^2 n - \alpha \rho n \]
\[ \dot{w} = D_n \nabla^2 w + \alpha_{w} \rho n - \beta_{w} w - \gamma \rho w \]
\[ \dot{c} = D_c \nabla^2 c + T[w,c] \rho - \beta_{c} c \]  

(1)

Here \( \rho \) and \( \rho_n \) are densities of motile and non-motile bacteria, \( c \) is a concentration of respiratory waste products, \( w \) is the chemotactic agent which is emitted by the bacterium, and \( n \) is nutrient which is eaten by bacteria. The functionals \( T \) and \( I \) are thresholds to be discussed below. We have used the freedom to rescale to set some coefficients to unity.

This equation contains a standard growth term which we typically take to be of the form \( G = \tau \rho^2 n/(n + n_0) - \rho^3 \), with \( \tau \approx 1 \); this form reflects the nutrient-inhibited growth of cells at low food concentration and finite reproduction rate which is achieved in a nutrient-“rich” limit. At high density of bacteria the growth is limited by the nonlinear term \(-\rho^3\). Motion is governed by diffusion and by a chemotactic term representing the response of the bacteria to a gradient in the attractant. As already mentioned, it will be important to incorporate the fact that sufficiently far behind the advancing front, the bacteria differentiate into a non-motile form. We assume that the transition occurs due to the accumulation of effects due to starvation; specifically, the transition to the non-motile phase occurs when \( n_n \equiv \int_0^t (n_0 - n) \delta(n_0 - n) \, dt \) exceeds some threshold value \( n_{tr} \), i.e. by setting \( I[w] = \delta \Theta(n_c - n_{tr}) \) (\( \Theta(x) \) is the Heaviside function). The rate of waste accumulation is proportional to the nutrient consumption term, and we assume that the waste decomposes with a rate dependent on the bacterial density, as this is in fact the underlying reason for the bacteria to aggregate. Finally, the emission of chemotactic agent \( c \) is proportional to the local density of bacteria, and it is triggered by local waste field; specifically, we assume that the chemotactic agent is emitted if either \( w > w_0 \) and \( c > c_0 \) or \( w > w_1 \), where \( w_1 > w_0 \) (formally, \( T[w,c] = \delta \Theta(w - w_0) \Theta(c - c_0) + \Theta(w - w_1) \)). The difference between \( w_1 \) and \( w_0 \) represents possible autocatalytic behavior of the attractant.

To understand the structure of our model, it is convenient to first consider the case of constant uniform nutrient \( n \gg n_0, \alpha = 0 \) and in the absence of any waste effects (i.e. \( T = 1 \)). The system now has an unstable steady-state \( \rho = c = 0 \). If we start with an initial condition of localized bacteria density amidst a sea of \( \rho = 0 \), the density will spread. In the absence of coupling to \( c \), the front would move at a speed of \( 2 \sqrt{D/\rho} \) via the usual marginal stability criterion [14]. By continuity, the front will continue to expand as long as \( v_e \) is not too large. On the other hand, the non-trivial uniform state is given by \( \rho = \sqrt{v_e}, c = 1/\beta \). The stability of this state to perturbations with wave vector \( q \) is given by the roots of

\[ (\omega + D_{\rho} q^2 + 2 \tau)(\omega + D_{c} q^2 + \beta_{c}) = v_e q^{1/2} q^2 \]  

(3)

It is easy to see that for large enough chemotactic response \( v_e \) this system has a band of wavevectors with purely real and positive growth rates \( \omega \). This is just the Turing instability described above. It does not require different diagonal diffusivities \( D_{\rho} \) vs. \( D_c \) but instead relies on there being a large cross diffusivity. Combining these arguments leads one to expect that generically there exists an intermediate range of \( v_e \) for which the bacteria density will propagate outward and create an expanding Turing-unstable region. Adding the nutrient field back in does not alter the above conclusions in any qualitatively important manner. The importance of waste dynamics will be discussed below.

To simulate the above equations, we used a split-step spectral code on a 128x128 lattice; specifically, the linear parts of the evolution were treated by FFT and the nonlinear pieces by explicit finite differences. Lattice anisotropy which comes primarily from the discretizing the nonlinear diffusion terms in eq. (1) was minimized by computing them simultaneously on two lattices which differ by 45° rotation [1]. Also, lattice discretization effects can lead to spurious (slightly) negative values of the density \( \rho \); this is presumably due to the lattice trying to mimic the possibility in the continuum of having precisely localized structures due to the nonlinear diffusivity [14]. To deal with this we used \( p > 1 \) (typically \( \approx 1.5 \)) and indeed it greatly reduced this spurious effect without any qualitative change in the dynamics. We can immediately verify the above simple features and proceed to discuss the formation of complex ordered structures.

Let us first focus on colony dynamics in the absence of a threshold for chemotactic emission \( T(w,c) = 1 \). In Figure 2, we show a snapshot of a typical simulation of the model equations. As the interface propagates outwards, it creates a set of concentric rings. The breakup of the rings into spots occurs somewhere behind the front, depending on the specific value of \( v_e \); for small enough \( v_e \), the rings do not break up. Ordered arrangements of the spots are hard to achieve, since the interaction between different rings is extremely small. Moreover, the appearance of a number of rings behind the outer rings is not in accord with the Budrene and Berg experiment. In most cases the spots appeared right behind the outer ring.

To account for this effect, we impose the threshold for chemotactic emission. The existence of this strong nonlinearity makes the rear part of the outer ring extremely sensitive to the structure of the waste and chemical fields. In particular, the emission is triggered preferentially at certain angular positions as opposed to along an entire ring. This then can cure the two previous problems - proper thresholding can lead directly to spots and previous spots strongly bias the formation of new spots. In fig. 3, we show the results of full model simulations,
showing significantly improved agreement with experiment. We now discuss in turn the threshold choices that lead to the differing results in figs 3.

In figure 3a, we see that spots form at positions such that they will eventually form radial rows; these rows are quite similar to those in the experimental pattern in Fig. 1a. The reason for this is that the concentration of $c$ in the ring of currently aggregating spots is nonuniform and due to diffusion this inhomogeneity spreads into the rear part of the outer ring. This biases the location for the new spots so they are created right in front of the previous spots. Once the spots form, they are not allowed to move very far since as nutrient depletes, bacteria transform into a non-motile phase. If the chemotactic response ($v_c$) is reduced, instead of radially aligned spots radial stripes are formed; this too has been seen experimentally (see Fig. 1b and 3b). We note in passing that the brightness of spots in experimental images is greater for motile bacteria so in all numerical figures we show the gray scale plots of $(p + 0.5\rho)$.

In the other main experimental spot pattern, the spots arrange themselves in a manner such that the new spot appears roughly between two pre-existing ones at the previous ring. By adjusting the values of $c_0$ and $w_1$ we can produce a similar pattern as well (see fig. 3c). By significant increase of the threshold $c_0$ we effectively made the residual level of chemoattractant irrelevant, and instead the waste threshold $w_1$ plays the major role. We should note, however, that this structure seems to be missing the visually striking spirals which create the sunflower impression in the original BB figure (fig 1a. of their paper). Whether this difference represents a shortcoming of our model which tends to keep spots along circular rings, or, as we feel more likely, that a larger system with less anisotropy and perhaps a different set of parameters could reproduce this visual impression is a question for future work.

To summarize, we have shown how the interplay of front propagation and a Turing-type instability can lead to spot patterns similar to those observed in bacterial aggregation. We have concentrated on generic mechanisms to spot patterns similar to those observed in bacterial aggregation. We now discuss in turn the threshold choices that lead to the differing results in figs 3.

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To summarize, we have shown how the interplay of front propagation and a Turing-type instability can lead to spot patterns similar to those observed in bacterial aggregation. We have concentrated on generic mechanisms which should remain important regardless of the details of the explicit biological interactions as these details become clearer. One important conclusion is that the biological mechanism for the “turning on” of chemotaxtrac-
tant emission is of critical importance in giving rise to the observed colonies. Of course, attempts at more quantitative comparison would require as input the functional dependencies of all the various pieces entering into the dynamics. We feel that this level of detailed modeling is a reasonable undertaking only after a demonstration that physics does indeed have a hope of describing what is going on without the need to invoke an incredibly complex hierarchy of biological mechanisms. This paper has been attempt to demonstrate exactly this point and to thereby provide motivation for a future quantitative study.

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12. The idea of using $p > 1$ to account for the discrete character of an aggregating particle (here a bacterium) arose in the context of diffusion-limited aggregation; see E. Brener, H. Levine and Y. Tu, *Phys. Rev. Lett.* 66, 178 (1991).
13. Strictly speaking, the integrated nutrient deficit should be done in “Lagrangian” variables, i.e. finding the nutrient deficit for individual bacteria as they move through space. This is hard to implement in our “Eulerian” description and in any case should be a small effect since in our model bacteria do not move over distances over which there are significant changes in nutrient concentration.
14. W. Van Saarloos, *Phys. Rev.* A39, 6327 (1989) and references therein. This actually applies only to the case $p = 1$, but as long as $p$ is not too large, the selected velocity changes only slightly from the marginal stability prediction.
15. The effect of residual anisotropy was determined by letting the system evolve for some time, rotating the fields by an irrational angle, and then continuing the time evolution. This procedure did not interfere with the radial row structure discussed later in the text.
16. For some results regarding nonlinear diffusion equations, see G. I. Barenblatt, *Similarity, self-similarity, and intermediate asymptotics* Consultants Bureau, New York (1979).

**FIG. 1.** Two examples of patterns of bacteria *E. coli* in experiments by Budrene and Berg [1]: radial alignment of spots (a), radially oriented stripes (b)
FIG. 2. A snapshot of bacteria density \((\rho + 0.5\rho_n)\) at \(t = 18\) within the model with \(D_n = 0.4\), \(D_c = 0.2\), \(D_w = 0.4\), \(\alpha = 0.12\), \(r = 1.8\), \(\beta_c = 1\), \(\alpha_w = 0.3\), \(\beta_w = 0.1\), \(\gamma = 0.2\), \(n_0 = 0.5\), \(n_{tr} = 0.03\), \(\delta = 0.5\), \(v_c = 6.0\), thresholds for chemoattractant disabled, system size is 40, time step 0.02. Concentric rings are formed in the wake of the outer ring.

FIG. 3. (a) Same as in Figure 2 but with enabled thresholds \((w_0 = 0.3, w_1 = 0.55, c_0 = 0.04)\) and \(v_c = 7\); radial rows of spots are clearly seen (cf. Figure 1a); (b) Same as in a but with weaker chemotaxis \((v_c = 6)\), radial stripes similar to Figure 1b are seen; (c) Pattern of \(\rho + 0.5\rho_n\) within the model with different thresholds \((w_0 = 0.3, w_1 = 0.5, c_0 = 0.5)\) and higher chemotaxis \((v_c = 9)\), other parameters are the same as in b. A staggered alignment is seen far from the center.
