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Patterns that Grow (in a dish)

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Abstract

Chemotactic bacteria have been observed to congregate into highly regular patterns. When the bacteria are placed in the center of a dish, a wave of bacteria can travel outward, leaving a regular pattern of spots or stripes in its wake. Although chemotaxis and excretion of an attractant can readily cause a pattern forming instability from a uniform state, they are not capable of generating patterns starting from a single spot. These patterns are apparently formed with the help of bacterial growth and depletion of nutrients in the growth medium.

Recent experiments have found that under certain special conditions, bacteria will form very regular patterns in a Petri dish [Budrene & Berg, 1991]. These patterns include equally spaced radial stripes, radial columns of spots, sunflower-like arrays of spots, and spots with radial tails arranged in chevrons. Bacteria were initially added to the center of the dish, which contained a growth medium suspended in semi-solid agar. The patterns formed in the wake of a circular "wave" radiating from the center of the dish. Less regular patterns could be obtained starting from bacteria spread uniformly in a thin layer of liquid. It was shown that the bacteria are capable of excreting aspartate, to which bacteria are strongly attracted.

It is obvious that if cells swim towards an attractant that they themselves excrete, the cells will tend to form clusters, since once cells become over abundant in one region, nearby cells will be attracted to that same region. In fact, this may be the most intuitive example of a uniform state that is unstable to perturbations that break the spatial symmetry. Yet we shall see that getting patterns of the type observed is not as easy as one might think. This will lead to ideas about why the bacteria are behaving in such a peculiar way.

A simple model

As is typical in pattern formation problems, determining exactly what pattern will form depends on nonlinearities in the problem, but a lot can be learned from a linear analysis as well. So, let us begin with the linear stability analysis of the simplest possible model for such an instability:

$$\begin{aligned} \dot{B} &= D_B \nabla^2 B - R\nabla \cdot (B \nabla A) \\ \dot{A} &= D_A \nabla^2 A + KB - \mu A \end{aligned} \qquad (1)$$

Here B is the concentration of bacteria, A the concentration of attractant, D_B and D_A are diffusion coefficients, R measures the attractiveness of the attractant, K is the rate at which bacteria excrete the attractant, and μ determines the lifetime of A. Equations of this type have been considered in the context of embryonic bone formation [Oster & Murray, 1989]. In what follows, μ will be assumed to be negligibly small.

The only nonlinearity in equation (1) is the quadratic $B\nabla A$ term. By linearizing about a uniform steady state with a concentration B_0 of bacteria, one finds that the system is neutrally stable to uniform perturbations and unstable to long wavelength perturbations, i.e., those with wavenumber k such that $0 < k < \sqrt{RKB_0/D_B D_A}$. The maximally unstable mode is near the middle of the unstable band and grows with a rate proportional to $(D_B +$

$D_A)RKB_0/D_BD_A$. That R and K appear together in the numerator of these expressions was to be expected, since they determine the rates of the two processes which are intuitively responsible for the instability. We can conclude that our intuition was correct, and that as long as the physical dimensions of the system (e.g., the size of the Petri dish) is larger that $\sqrt{D_BD_A/RKB_0}$ then bacteria in an initially uniform state will form a pattern, consistent with the experiments done in the liquid medium.

The fact that R and K appear in the above expressions only as the product RK has an interesting interpretation which may be of some biological relevance. Because only the product appears, the linear analysis will give exactly the same results if each constant is multiplied by -1. If R is negative, then A is a repellent, not an attractant. If K is negative, then A is destroyed by the bacteria, not created. Thus, at least at the linearized level, being repelled by something you destroy is equivalent to be attracted to something you make. Either situation results in congregation.

Indeed, being repelled by something the bacteria destroy has been proposed as the underlying reason for these patterns [Budrene & Berg]. Since the carbon source used in the experiments is one of the more oxidized intermediates of the Krebs cycle, it is possible that the bacteria are in a state of oxidative stress. Since the bacteria use up oxygen, it makes sense that by clustering together the bacteria could lower the local oxygen concentration and thereby relieve their stress. The intuition for pattern formation is the same as before, but vice versa: fluctuations that lower the concentration of bacteria in a region result in fewer bacteria in that region and hence more repellent, and so forth. If the oxidative stress idea is correct, it means that bacteria are making use of this symmetry. Rather than swimming away from the oxygen they consume, they simply generate an attractant and swim towards it. The effect is the same.

Patterns from a point

Understanding the patterns that form from a single initial spot is a bit more difficult; for one thing, we cannot perturb about a uniform steady state. Let us begin by carrying out a dimensional-type argument. For a spot to be at a steady state, the flux outward due to diffusion must be offset by the flux inward due to the attractant:

$$D_B\nabla B = RB\nabla A. \qquad (2)$$

At steady state, the outward flux of A must be balanced by excretion:

$$D_A\nabla A = D_BB_T/r^{d-1}, \qquad (3)$$

(ignoring multiples of π) where r represents the radius of the spot, B_T is the

total amount of bacteria in the spot, and d is the number of spatial dimensions in the problem. Putting these two equations together and estimating ∇B as B/r we find that

$$r^{d-2} = \frac{RKB_T}{D_B D_A}. \quad (4)$$

In one dimension ($d = 1$) this says $r = D_B D_A / RKB_T$, and we can put this equation to the test because the equation for a steady state is exactly soluble. Substituting $B = -D_A \nabla^2 A/k$ yields:

$$-D_B \nabla^4 A - R\nabla(\nabla^2 A \nabla A) = 0 \quad (5)$$

which can be repeatedly integrated, and the solution for B is

$$B(x) = \frac{RKB_T^2}{8D_B D_A} \mathrm{sech}^2\left(\frac{RKB_T x}{4D_B D_A}\right). \quad (6)$$

We see that our estimate for the size of the spot was quite good. Also, we have learned that in one dimension, a steady state can have at most one spot. However, since the spots decay exponentially we can assume that a state with several spots very far apart will take a very long time to relax to a single spot.

Peculiarities of 2D

In two dimensions, the situation is different. First of all, the equation for a steady state does not readily yield exact solutions. Secondly, our equation for the radius of the spot is obviously meaningless. If we carry out the same argument again, taking into account that all the rs cancel, we realize that a steady state should occur only when B_T, the total amount of bacteria, equals a certain value, proportional to

$$B_T = \frac{D_B D_A}{KR}. \quad (7)$$

If there are more than this many bacteria in a spot, it should collapse to a singularity. If there are fewer, the spot will spread.

If the spot spreads, we might hope that it will spread into a fairly uniform state, which would then be ripe for undergoing pattern formation as discussed above. How far does it have to spread for this to happen? The size of the spot must be bigger than the critical wavelength of $\sqrt{D_B D_A / RKB_0}$, and in fact should be much bigger if we are to get any kind of non-trivial pattern. Naturally B_0 will go as B_T/r^2, so we find

$$r \gg \sqrt{\frac{D_B D_A}{RKB_T}}\, r \quad (8)$$

or,

$$\frac{RKB_T}{D_B D_A} \gg 1. \quad (9)$$

Thus, there is *no* r at which patterns begin to form! If the quantity $RKB_T/D_B D_A$ is bigger than one, we already found that the single initial

spot will collapse, not spread. If it is less than one, the initial spot will spread and keep on spreading. This has been confirmed by numerical simulations, starting with a Gaussian spot. Depending on the initial shape, it is possible to get pattern formation within the original spot, but it is not possible for the spot to spread and leave a pattern in its wake, as seen in the experiments.

What makes the wave?

We see that the formation of patterns of isolated spots starting from a single central spot requires more than just changing the initial conditions of a system whose uniform state is unstable. Thus it appears likely that the experiments with bacteria involve more than just attraction towards an excreted molecule.

More evidence that this process cannot be described by the equations (1) alone comes from the observation that the circular wave that travels outward from the center of the dish moves at a constant velocity. Such wave-like solutions in diffusive media are typically fronts at the boundary between two quasi-steady states. In this case the steady state ahead of the wave front is obviously the absence of any bacteria. The steady state behind the front probably consists of bacteria whose growth has stalled due to depletion of some nutrient in the growth medium.

This is consistent with the fact that the patterns seem to have the same fundamental wavelength everywhere; the spacing of spots or stripes is the same at the outer edge of the dish as it is near the center. From our experience with equation (1), this hints that the concentration of bacteria in the wake of the wave is unchanged as the wave travels outward, since the critical wavelength depends on the concentration of bacteria. The concentration of bacteria in the wake would indeed be constant if the wave represents a transition from no bacteria to bacteria whose growth using the more readily metabolized nutrients has saturated.

This idea makes sense in terms of the biochemistry of the Krebs cycle as well. In the experiments, the bacteria excrete large amounts of aspartate; an intracellular concentration of 0.2M would be necessary if the bacteria are simply excreting aspartate they have stored. Thus, it is likely they are making it from the Krebs cycle intermediates that are abundant in the growth medium that was used. When the leucine and threonine in the growth medium are used up, the bacteria will be unable to catabolize the remaining amino acids according to the normal Krebs cycle. Under these conditions, one would expect oxaloacetate to accumulate in the cell, and this would tend to cause creation of aspartate through the ac-

tion of aspartate-glutamate transaminase [Lehninger, 1970]. On the other hand, when leucine or threonine is abundant, one expects bacteria to consume any available aspartate.

Presumably, the bacteria grow much faster when leucine or threonine is available than when not, and while growing they will consume these amino acids rapidly. Considering these two processes plus diffusion leads us to consider the equations:

$$\begin{aligned} \dot{B} &= D_B \nabla^2 B + K_{\rm grow} B L \\ \dot{L} &= D_L \nabla^2 L - K_{\rm eat} B L \end{aligned} \qquad (10)$$

where L is the concentration of leucine or threonine (or their sum). This set of equations does admit wave-like solutions such as those seen in the experiments. Ahead of the wave, B is zero; behind it, L is (nearly) zero. In fact, in the special case where $K_{\rm grow} D_L = K_{\rm eat} D_B$ then $L = L_0 - K_{\rm eat} B / K_{\rm grow}$ and one has

$$\dot{B} = D_B \nabla^2 B + K_{\rm grow} B L_0 - K_{\rm eat} B^2, \qquad (11)$$

with L_0 the initial concentration of leucine. This is known as Fisher's equation, and has propagating wave solutions with a constant speed equal to $\sqrt{4 D_B K_{\rm grow} L_0}$ [Fisher, 1937].

The value of D_B was estimated by the experimenters to be 4.8×10^{-6} cm^2/sec, and since the medium is designed to saturate the rate of growth, $K_{\rm grow} L_0$ should be in the range of the maximal growth rate for bacteria. Taking a doubling time of 30 minutes, we get an estimated wave speed of 8.6×10^{-5} cm/sec. This estimate is only a factor of four larger than the wave speed of 2.1×10^{-5} cm/sec quoted in the experimental paper. Thus it is plausible that bacterial growth and nutrient depletion are the main factors responsible for the observed wave.

The experiments done with a prestirred bacteria/growth medium mixture showed that patterns could form in less than a generation time, and that therefore growth was not necessary for pattern formation. This is consistent with the ideas presented here. Getting patterns to form from a uniform state is "easier" than getting them from a single initial spot. It is likely that the traveling wave, which depends on growth, sets up conditions in its wake that are similar to those in the stirred medium. Once these conditions are established, the attractive instability takes over and patterns form.

The figures show some results of numerical simulations of model equations. Many models were tried; the most convincing patterns formed in those that had bacteria, aspartate, and "leucine" (which could really be any nutrient in short supply) as the main variables. In all of the models that produced a wave, bacteria grew and ate leucine as in the above equation, and produced aspar-

tate in the presence of succinate, which maintained a nearly constant concentration. Models where the bacteria were chemotactically attracted to both aspartate and leucine gave waves where the bacteria concentration was higher near the wave front than in the wake, as was also seen in the experiments.

In no model has the striking geometric regularity of the bacterial patterns been seen. This could be due to a tendency to use parameter values that will allow the wave to be followed an appreciable distance without using vast amounts of computer time. Forcing the patterns to form quickly probably makes them more random. Of course, pattern selection depends very much on nonlinearities. Some of the nonlinearities in this problem—such as receptor saturation—can be (and were) readily modeled at a qualitative level, but there are likely to be nonlinear effects in the chemistry (think of many enzymes, especially the allosteric ones, in the Krebs cycle) and elsewhere in the system that even the most ambitious model would fail to capture. Still, the simple analysis that was presented here leads to some suggestive possibilities in terms of why the bacteria are doing what they do. The question of whether the bacteria are being smart and avoiding stress or just making patterns for no useful reason at all remains unanswered.

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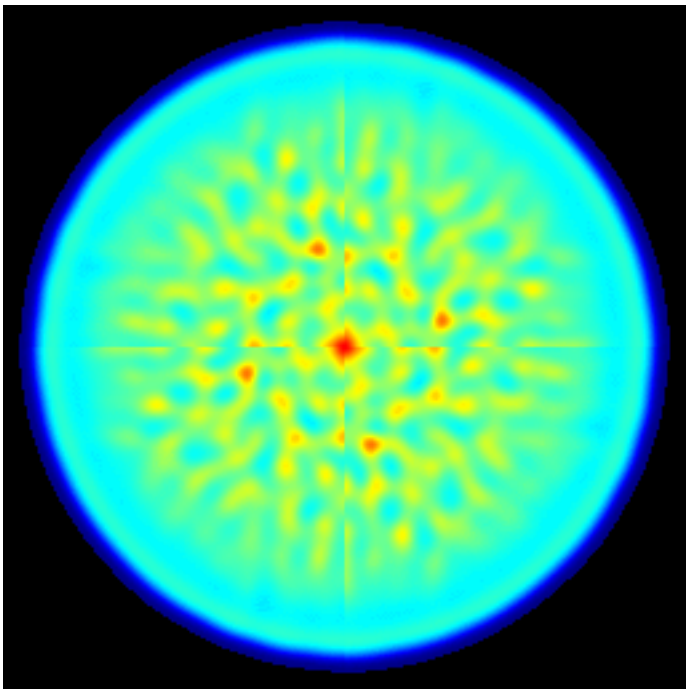

Figure 1.: A simulation of a pattern forming from a small spot. The lighter, circular region denotes higher bacteria concentration. The region gets larger as time progresses. This image was converted from color; the small dark spots in the bright region actually contain more bacteria than the surroundings, while the black outer region contains almost no bacteria. This figure and the others were made by pasting together four copies of a simulation of a quarter circle wedge.

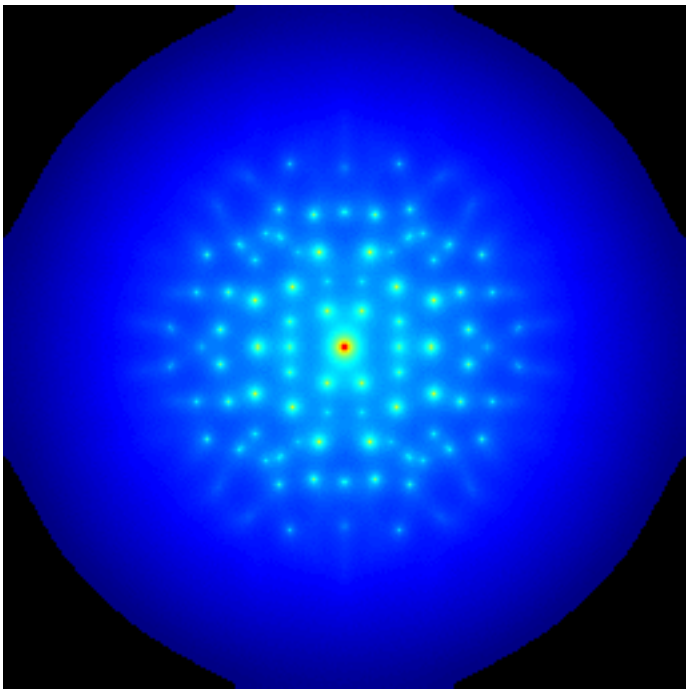

Figure 2.: With these parameters, the spots are more localized, as in the real experiments, but the arrangement of the spots seems fairly irregular compared with what the real bacteria did.

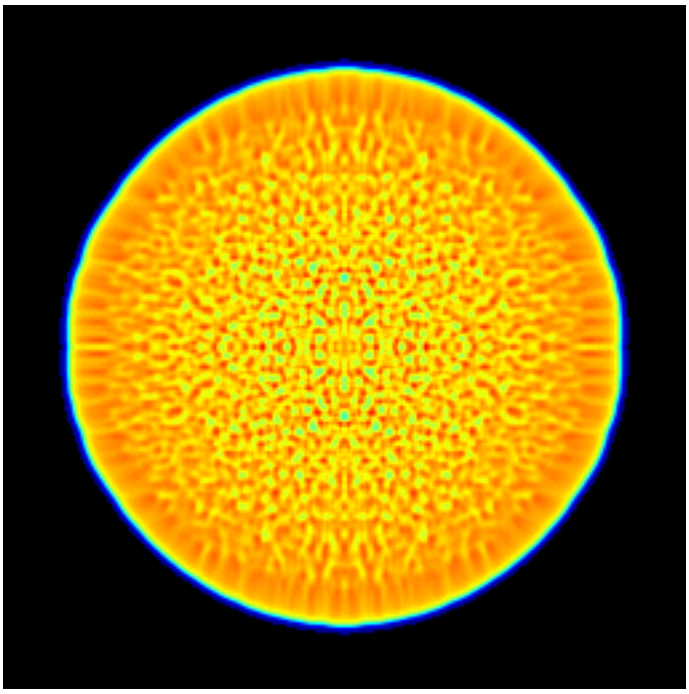

Figure 3.: In this simulation, the bacteria were chemotactically attracted to the leucine as well as the aspartate. The predominant wavelength shorter because a different scale was used.