Cooperative organization of bacterial colonies: from genotype to morphotype.

by Eshel Ben-Jacob, Inon Cohen and David L. Gutnick

The mode and dynamics of cooperative organization which leads to complex pattern formation in bacteria are described. A generic modeling method was used to study the culture behavior and dynamics during colonial development. The results showed the use of cooperative cellular interactions during the process of self-organization. Various cell-cell signalling such as long-range chemorepulsion, short-range chemoattraction and rotational chemotaxis are described.

INTRODUCTION

The endless array of patterns and shapes in nature has long been a source of joy and wonder to laymen and scientists alike (76). Discovering how such patterns emerge spontaneously from an orderless and homogeneous environment has been a challenge to researchers in the natural sciences throughout the ages. Many phenomena display the emergence of patterns during diffusive growth, ranging from the growth of snowflakes to solidification of metals, from the formation of a coral reef to cell differentiation during embryonic development.

In the early 1950s, Alan Turing understood that patterns would evolve in systems driven out of equilibrium, where competition and interplay between various tendencies exists (81). We now understand that the diffusion field drives the system towards decorated (on many length scales) irregular fractal shapes. It has become clear that the competition between the drive of the diffusion field and a reverse, stabilizing, drive of microscopic effects (e.g. surface tension and surface kinetics) plays a key role in the determination of the evolved pattern.

Here we describe cooperative patterning during growth of bacterial colonies under hostile conditions of low level of nutrients, a hard surface, or both. Under such conditions, not unlike certain ecosystems in natural environments, complex colonial patterns are observed (10, 11, 14, 18, 40, 58, 59, 61, 63, 74). Drawing on the analogy with diffusive patterning in nonliving systems (5, 8, 49, 54) the above observations can be understood as follows: The cellular reproduction rate, that determines the growth rate of the colony, is limited by the level of nutrients available for the cells. The latter is limited by the diffusion of nutrients towards the colony (for low nutrient substrate). Hence, the colonial growth should be similar to diffusion limited growth in nonliving systems, such as solidification from a supersaturated solution, growth in a Hele-Shaw cell, electrochemical deposition, etc (5, 8). Indeed, for some conditions bacterial colonies can develop patterns reminiscent of those observed during growth in nonliving systems (10, 11, 14, 18, 40, 58, 59, 61, 63).

In general, the bacteria can exhibit richer behavior than abiotic patterning, reflecting the additional levels of complexity involved (10, 12-16, 21, 33). In the former case, the building blocks themselves are living systems; each has its own autonomous self-interest and internal degrees of freedom. At the same time, efficient adaptation of the colony to adverse growth conditions requires cooperative behavior of the bacteria. The bacteria can do so because they possess various modes of communication, such as (a) direct cell-cell physical and chemical interactions (34, 64); (b) indirect physical and chemical interactions, e.g. production of extracellular "wetting" fluid (44, 62); (c) long range chemical signaling, such as quorum sensing (41, 42, 55); and (d) chemotactic signaling [chemotactic response to chemical agents that are emitted by the cells (27, 30, 31)].

Studies on pattern formation in abiotic systems demonstrated that different shapes are observed for the same system as the control parameters are varied (e.g. undercooling, supersaturation). Although a number of morphologies are possible under a particular set of conditions, only one is generally observed ("selected"). The commonly accepted morphology selection principle states that the particular morphology selected is the fastest growing one (8, 9). Hence the observed patterns can be organized in a morphology diagram analogous to a phase diagram (of liquid, solid, gas). There is a relatively sharp transition from one shape to the other, as the control parameters are varied and different morphologies are selected.

It has been demonstrated that the concept of morphology diagram can also be applied to the growth of bacterial colonies (6, 10, 12, 14, 59, 61, 63), i.e. the patterns exhibited by a given strain can be organized as a mosaic of regimes (each for a characteristic pattern) on a graph of nutrients and hardness of agar. The sharp transitions between the various regimes imply that at each regime, a characteristic biological feature dominates the growth.

Cooperative organization of bacterial colonies: from genotype to morphotype.

In Figure 1 we refer to an additional concept introduced to describe the patterning of bacterial colonies. Three patterns with different geometrical characteristics are shown. The first is best characterized by its branching pattern. In the second, the branches are much thinner and all have a twist with the same handedness, while in the third every branch has a leading droplet consisting of many bacteria at its tip. Microscopic observations (described below) reveal that the dynamics at the cellular level are also very different in each of the patterns. In addition, these geometrical characteristics are inheritable. In order to describe the distinctive characteristic properties of each colonial development, a new concept was introduced: morphotype. The patterns in Figure 1 are representative patterns of the tip-splitting (T), chiral (C), and vortex (V) morphotypes. It should be noted that different bacterial strains (and species) can belong to the same morphotype. Moreover, as we show below, bacterial strains can undergo a transition from one morphotype to another (e.g. branched to chiral). The morphotype transition requires a period of adaptation on the order of days and once the transition has occurred, the new morphotype is stable. It is clear that both pattern formation and morphotype transition require a mode of organized intercellular communication and cooperative multicellular behavior, both of which are characteristic features of a variety of cooperative and cell-density dependent physiological processes currently under study in microbial physiology (42, 48, 56).

THE T AND C MORPHOTYPES

The patterns illustrated in Figures 2-4 were formed by organisms that were initially isolated from cultures of Bacillus subtilis (10, 13). 16S RNA sequence analysis in combination with a number of phenotypic and biochemical characterizations was used in the identification of these strains as members of the genus Paenibacillus (M Tcherpikov et al, unpublished information) and assigned to a new species, Paenibacillus dendritiformis. The strains in these figures belong to two different morphotypes, C and T (7, 17, 19). They showed greater than 99% sequence similarity to each other.

P. dendritiformis exhibited a profusion of patterns as the growth conditions were varied [ILLUSTRATION FOR FIGURES 2-4 OMITTED] (12, 14). Observations of similar patterns during growth of other bacterial strains have been reported (57, 61, 63, 67). Under the microscope, cells were seen to swim in a characteristic random-walk-like fashion within a fluid. This fluid seems to be excreted by the cells, although it could also be drawn from the agar during microbial growth (12, 13). The swimming was confined to the fluid; isolated cells spotted on the agar surface did not move. The boundary of the fluid thus defines a local boundary for the branch of the developing colony. Whenever the cells are active, the boundary propagates slowly as a result of the cellular movement and production of additional wetting fluid. The observations also revealed that the cells were more active at the outer parts of the colony, while closer to the center the cells did not move, and some were observed to sporulate.

The C morphotype also exhibited a morphology diagram with a profusion of most beautiful and complex patterns [ILLUSTRATION FOR FIGURE 3 OMITTED] (13, 16, 17). In addition to the enormous difference in morphologies between the genetically similar variants, microscopic observations indicated that the cells were longer than those of the T morphotype. Electron microscopic observations indicated that the chiral structure did not stem from twisting of the cell membrane (13). The question then arises whether there is a connection between the increased length of the cells and the formation of chiral patterns.

Chiral asymmetry, first discovered by Louis Pasteur, exists in a whole range of scales, from subatomic particles through human beings to galaxies, and seems to have played an important role in the evolution of living systems (2, 45). Bacteria display various chiral properties. For example, Mendelson et al (64-66, 68) showed that long cells of B. subtilis can grow in helices in which the cells form long strings that are twisted around each other.

Since, in the case of P. dendritiformis chirality is formed by moving swimming cells, a different mechanism is controlling the handedness. One possibility may have to do with the specific handedness associated with flagellar rotation (37, 73, 77). Ben-Jacob et al have proposed that it is this property of flagellar handedness, coupled with strong cell-cell orientation

interactions, that accounts for the observed chirality (16).

Morphotype Transitions

When plated on soft agar (concentrations of about 1% or less) colonies of T cells develop with characteristic tip-splitting morphology. Occasionally, after a period of about 48 h, a morphotype transition occurs, reflected in bursts of growth of C morphotype. Cells of C morphotype can be isolated from such bursts (14, 16). In these growth conditions colonies propagate faster then those of the T cells, reaching the same distance from the spot inoculum with significantly fewer bacteria. Motivated by the "fastest growing morphology" selection principle for nonliving systems, Ben-Jacob et al proposed that the colonial

Cooperative organization of bacterial colonies: from genotype to morphotype.

growth velocity (the rate of spreading) is the colonial selective pressure leading to the T [right arrow] C morphotype transitions [ILLUSTRATION FOR FIGURE 3 OMITTED]. If this hypothesis is correct, one would expect to observe the reverse C [right arrow] T transitions under those growth conditions for which T spreads fastest. Indeed such reverse transitions are observed during growth on harder agar where T spreads faster than the C morphotype (10, 14).

The T [right arrow] C transitions are quite frequent. Under proper conditions, about 60% of T colonies show bursts of the C morphotype after about two days. The question arose as to whether this was a true morphotype transition, or whether the C cells were simply present as a small minority within the var. dendron population. Recent findings using strains marked with antibiotic resistance markers have demonstrated that under the specific conditions, the cells undergo an actual morphotype transition (M Tcherpakov et al, manuscript in preparation). Moreover, morphotype transition appears to be a more general phenomenon. For example, at least two different isolates of P thiaminolyticus from various collections have also been shown to exhibit characteristics of both the T and C morphotypes, albeit under slightly different conditions of nutrient concentration and agar hardness. Moreover, these strains also undergo the same mode of morphotype transition (M Tcherpakov et al, in press).

MODELING AND SIMULATIONS OF THE COLONIAL DEVELOPMENT

While the modeling of a biological process can be limited to finding a suitable formulation to simulate the process using a computer, ideally one would like to have a generic model that not only leads to a numerical simulation, but also incorporates features that are testable experimentally. In addition, the model should contain elements that allow for a variety of hypothetical situations that might also lead to unanticipated experimental predictions.

The Communicating Walkers Model

To model the colonial development of the T and C morphotypes, Ben-Jacob et al (12, 13) developed "the communicating walkers model." The bacteria are represented in the model by particles dubbed "walkers," which correspond to about [10.sup.2]-[10.sup.4] bacterial cells.

A walker is specified by its location on the surface and its metabolic state (referred to as an "internal energy"). The latter is increased by consumption of nutrients from the media and subsequently used in order to drive the walker's (bacterial) activities and metabolic processes. For high concentration of nutrients, the food consumption is higher then the spending rate. Hence, the internal energy increases until it reaches a threshold level at which the walker divides. When there is not enough "food," the walker can consume only the available amount, which can be lower than needed for activity. As a result, the internal energy decreases until it drops to zero. The walker then becomes immotile and remains in this state (enters prespore state). In the model, we assume for simplicity a single component of food, which satisfies a simple diffusion equation. As the walkers consume the nutrient, the concentration decreases in front of the colony and additional nutrient diffuses towards the colony. Hence, it is a diffusion-limited growth, as mentioned above.

In laboratory experiments, bacteria swim within the lubrication fluid. In the model, the walkers perform a random walk. At each time step, each of the active (motile) walkers moves a step at a random angle. The walkers are confined within an envelope (defined on a tridiagonal lattice) which represents the boundary of the lubrication fluid. In the event a walker's step would lead to its movement outside the boundary, the step is not performed, and a counter on the appropriate segment of the envelope is increased by one. When a segment counter reaches a threshold [N.sub.c], the envelope segment propagates, adding one lattice area to the colony. This requirement of [N.sub.c] hits represents the colony propagation through wetting of unoccupied areas by the bacterial cells. This feature reflects the local cooperation in the behavior of the bacteria (the analog of a surface tension in nonliving systems). Note that, to a first approximation, [N.sub.c] represents the agar concentration, since more "collisions" are needed to push the envelope on a harder substrate. Results of numerical simulations of the model are shown in Figure 4.

To test the idea that both flagellar handedness and increased cell size play the major role in the origin of the chiral growth of the C morphotype, Ben-Jacob et al (16) included the additional features of flagellar handedness and cell-cell orientational interaction in the communicating walkers model. To represent the cellular orientation, each walker is assigned an orientation. Every time step, each of the active walkers performs rotation to a new orientation that is derived from the walker's previous orientation. Once oriented, the walker advances a step in either the forward or reverse direction (an experimental observation). As for the T morphotype, the movement is confined within an envelope that is defined on a triangular lattice. Results of the numerical simulations of the model are shown in Figure 4.

Cooperative organization of bacterial colonies: from genotype to morphotype.

The communicating walkers model described here is only one example of a modeling approach. There is another class of models, continuous models, in which the bacteria are represented by the value of their local density (10, 51, 59, 60).

CHEMOTAXIS-based ADAPTIVE SELF-ORGANIZATION

Chemotaxis is the best studied and most prevalent signal transduction system in motile bacteria (38). This process involves changes in the movement of the cell in response to a gradient of certain chemical fields (1, 22, 23, 53). The movement is biased along the gradient either in the forward (in the direction of the gradient) or in the reverse direction. Thus, chemotaxis enables microbial cells in a variety of natural environments to obtain more favorable conditions, such as movement towards nutrients, escape from predators, movement towards specific surfaces, and protection by cellular aggregation. Because space constraints prevent us from including a thorough analysis of the chemotaxis literature, we refer the reader to a number of recent excellent reviews of the field (26, 37, 43, 44, 78).

Usually chemotaxis implies a response to an externally produced field such as attraction towards supplemented nutrients. However, self-generated bacterial chemotactic signaling by the excretion of amino acids and peptides has also been demonstrated (27, 30, 31, 85). In the case of Escherichia coil and Salmonella typhimurium, this mode of chemoattraction involves membrane receptors such as the Tar receptor for chemotaxis, as well as a new receptor involving chemoattraction on rich medium (27).

At least 50 different gene products are involved in governing the mode by which microbes employ the chemotactic system to modulate their movement. The cell "senses" the concentration of the chemoattractant (or repellent) by measuring the fraction of receptors occupied by the signaling molecules. Thus, at very high concentrations the chemotactic response vanishes because of receptor saturation - the "receptor law." At the lower limit of attractant, the response is also negligible since it is "masked" by noise in the system. Swimming bacteria such as E. coli perform chemotaxis by modulating the time gap between tumbling events. Increasing (or decreasing) this time gap when swimming up or down the gradient of attractant (or repellent) bias their movement toward (or away from) favorable (unfavorable) locations. In E. coli for example, the tumbling event is controlled by the protein Che Y.-Phosphorylated, CheY binds to a switch at the base of the flagellar motor, thereby changing the flagellar rotation from counter-clockwise (the default direction of rotation, which propels the cells in a more or less straight trajectory), to clockwise rotation (which causes the cells to tumble). The signal transduction pathway involving the phosphorylation of CheY involves the action of an autophosphorylating kinase, CheA, whose activity is controlled by chemoreceptors. The product of CheA, CheY [approximately] P, is then dephosphorylated by the action of CheZ, a phosphatase whose action depends on its interaction with CheY [approximately] P. Interestingly, an additional mode of regulation has been discovered involving the oligomerization of CheZ, which apparently is mediated by its interaction with CheY [approximately] P (28). It is the oligomer that catalyzes the dephosphorylation of CheY [approximately] P. In other swimming species, the details of regulation and signal transduction may vary, but the mechanism of chemotaxis via modulated tumbling time is conserved.

Ben-Jacob et al (19, 32) assumed that for the colonial adaptive self-organization the T morphotype employs three kinds of chemotactic responses. One is the nutritional chemotaxis mentioned above. According to the "receptor law," it is expected to be dominant for a range of nutrient levels. The two other modes of chemotaxis are self-induced, that is there is chemoattraction or chemorepulsion towards or away from signaling molecules produced by the bacterial cells themselves. As we show below, for efficient self-organization it is useful to employ two chemotactic responses operating on different length scales, one regulating the dynamics within the branches (short length-scale) and the other regulating the organization of the branches (long length-scale).

The observations of attractive chemotactic signaling in E. coli (15, 27, 30, 31, 80) indicate that it operates during growth at high levels of nutrients. Motivated by the above, we assume that the colony employs an attractive, self-generated, short-range chemotaxis during growth at high levels of nutrients. To test this hypothesis, we add the new feature to the communicating walkers model and compare the resulting patterns with the observed ones. In Figure 4 we show an example of the formation of 3D structures when the attractive chemoresponse is included in the model.

In Figure 4 we also demonstrate the dramatic effect of the repulsive chemotactic signaling emitted by stressed walkers (12, 15, 19-21, 32). The pattern becomes much denser with a smooth circular envelope, while the branches are thinner and radial. This structure enables the colony to spread over the same distance with fewer walkers, thereby providing the developing colony with a distinct biological advantage under certain conditions of nutrient stress.

Cooperative organization of bacterial colonies: from genotype to morphotype.

PATTERN FORMATION IN ESCHERICHIA COLI AND SALMONELLA TYPHIMURIUM

Physiological Characterization

One of the most dramatic examples of cell-cell communication mediating pattern formation in swimming bacteria was demonstrated by Budrene & Berg (30). They showed that under conditions of energy limitation, cells of E. coli swimming in a thin layer of liquid substrate can form various patterns such as concentric rings, sunflower-like structures of spots, and radial arrangements of spots. They showed pattern formation only on minimal media in a Tar-dependent pathway. Spot formation was attributed to cellular aggregation, which in turn required the excretion by the cells of a chemoattractant signaling molecule(s) (aspartate or glutamate). The hypothesis was that cellular aggregation could provide a mechanism for reducing local oxygen concentrations, thereby protecting the cells from damage caused by free radicals and superoxides. Support for the hypothesis included the observation that the addition of [H.sub.2][O.sub.2] induced cell aggregation and triggered excretion of the chemoattractant.

A similar behavior of pattern formation was also reported in S. typhimurium by Budrene & Berg (31) and by Blat & Eisenbach (27). In the latter report, pattern formation was shown to occur not only on minimal media in a Tar-dependent pathway, but also on rich media such as tryptone or Luria broth. To date, this behavior has not been demonstrated in E. coli. The patterns formed on rich media were shown to be independent of the Tar receptor, although they were induced by [H.sub.2][O.sub.2]. In this regard, pattern formation in S. typhimurium was recently shown to occur in mutants defective in two major regulatory proteins that mediate cellular responses to oxygen stress, [Oxys.sup.R] and [Rpo.sup.s] (G Beck, personal communication).

The question remains regarding the significance of the additional pathway for pattern formation on rich media in S. typhimurium. One possibility is that since pattern formation in both E. coli and S. typhimurium is highly sensitive to small variations in agar thickness, temperature, etc, it may be that under more suitable conditions, motile strains of E. coli will also be shown to form patterns on rich media via an alternative pathway. It also remains to be determined whether the Tar-independent pathway in S. typhimurium is mediated by a new receptor, or whether under specialized conditions, a known receptor is "recruited" for the chemosignaling under this set of conditions.

Modeling the Patterns - Additional Processes

In all of the reports above, the patterns were attributed to aggregation of the cells as a response to chemoattractant signaling. It was not demonstrated that such a mechanism, triggered by oxidative stress, is sufficient to explain all emerging patterns. To test this hypothesis, Ben-Jacob et al composed a model (15, 80) similar to the one for the branching patterns described above. Again, the bacterial cells (E. coli or S. typhimurium in this case) are represented in the model by walkers that perform random walk, consume food to increase their internal energy, spend this energy for activity, reproduce when food is abundant, and become nonmotile as they approach starvation. In the experiments, the bacteria do not swim in a layer of fluid on top of the agar (as in the branching patterns), but rather swim inside it. Therefore, there is no sharp boundary to the bacterial colony, and in the model, no envelope is present. The addition of a diffusing attractant that is constantly emitted by the bacteria, together with bacterial chemotaxis towards its gradient, leads to creation of spots. Others (29, 80, 85) have reported the same conclusions, and it was verified (85) in an experiment wherein an addition of [H.sub.2][O.sub.2] induced the constitutive emission of attractant.

This standard approach is insufficient to explain several crucial observations (30). In the experiments, spots appear sequentially in the wake of a spreading broad ring and later "lock" into position as the bacteria turn nonmotile. To capture these effects, one must introduce additional mechanisms. First, Ben-Jacob et al (15, 80) explicitly include in the model a "triggering" field, i.e. a field whose concentration must reach a threshold before attractant emission is activated. Ben-Jacob et al proposed that the value of the threshold for emission may depend on the ambient chemoattractant concentration: This threshold is high if there is no chemoattractant, and the threshold is lower if the chemoattracrant concentration is high. This proposal means that both the oxygen metabolism and the attractant pathway affect the emission of the chemoattractant. In the absence of such an autocatalytic effect, the model cannot produce the heretofore observed radial structures. To observe (in the simulations) different patterns, Ben-Jacob et al vary the model's parameters related to the bacterial response to the triggering field and to the precise nature of the chemoattractant signaling. This version of the model is in good agreement with many of the features seen in experiment. The model has one drawback: It can produce radial organization of spots or stripes only when it includes a seemingly nonbiological response to chemoattractant.

One of the features required of a good model is a close relation with the biological knowledge. Therefore Ben-Jacob et al (15) claimed that a model with biological

Cooperative organization of bacterial colonies: from genotype to morphotype.

response to chemoattractant can produce radial organization of spots or stripes only with an additional element. They proposed that, as in the branching patterns, starved bacteria emit chemorepellent. Indeed, a model that includes this feature and chemotactic response to this repellent can account for all the salient features of the experiments. All the observed patterns can be obtained by changing the relative strength of the repulsive and attractive effects [ILLUSTRATION FOR FIGURE 5 OMITTED].

The results of the models led to the proposal that E. coli and S. typhimurium might employ the following (previously unreported) features as part of the response to energy depletion (15, 80): that (a) attractant perception affects its emission, and (b) repellant is emitted by starved cells.

Mutants Defective in Pattern Formation

Recently, Eisenbach et al (3) began a genetic study of pattern formation in S. typhimurium. Eight mutants were isolated that retained the ability to swarm and perform chemotaxis, but were defective in the Tar-dependent pathway for pattern formation [ILLUSTRATION FOR FIGURE 6 OMITTED]. No mutants were isolated that were defective in the Tar-independent pathway. Seven of the eight mutations were tentatively identified. Four of the eight mutants were mutated in a hypothetical reading frame termed yoj located just downstream of the gene ompC. The yoj ORF appears to encode a characteristic signal peptide for a lipoprotein and was found to be homologus to an inner membrane protein, ApbE, which is involved in biosynthesis of thiamin in S. typhimurium (4). To test the involvement of Yoj in pattern formation, the gene was cloned behind an inducible tac promoter and introduced into the yoj mutants defective in pattern formation. Complementation was demonstrated only in the presence of IPTG (3) [ILLUSTRATION FOR FIGURE 6 OMITTED]. In addition to the mutants in yoj, two additional mutations were mapped to the ilv operon, ilvG and ilvM, respectively. These two genes encode the two subunits of the enzymes acetohydroxy acid synthase II (AHAS II), which is involved in valine and isoleucine biosynthesis (71). Finally, an additional mutation was mapped to a gene that showed greater than 90% homology to the pfl locus in E. coli K-12. Pfl, pyruvate-formate lyase, catalyzes the conversion of pyruvate to formate under anaerobic conditions (70, 84).

The roles for these proteins in pattern formation are unknown at this point. One possibility is that mutations in the yoj and pfl genes are somehow related to oxygen metabolism and stress initiating the chemoattraction. In this regard, it has recently been shown (36) that lowering the flux of metabolites through the isoleucine-valine biosynthetic pathway induces a number of stress-response promoters controlled by RpoS. This could account for the selection of pattern forming mutations in the subunits of AHAS II. Further analysis of new mutants will be required in order to decipher the genetic basis of the phenomenon.

THE VORTEX MORPHOTYPE

Bacterial Patterns and Dynamics

More then half a century ago, observations of migration phenomena of Bacillus circulans on hard agar surface were reported (39, 75, 83). The observed phenomena include "turbulent like" collective flow, complicated eddy (vortex) dynamics, merging and splitting of vortices, rotating "bagels," and more. This behavior is not unique to B. circulans. During studies of complex bacterial patterning, new strains that exhibited behavior similar to B. circulans were isolated (14, 18). We refer to the new morphotype produced by these strains as the Vortex (V) morphotype [ILLUSTRATION FOR FIGURE 7 OMITTED]. The strain illustrated in Figure 7 has recently been shown to belong to the genus Paenibacillus (M Tcherpakov et al, manuscript in preparation).

A wide variety of branching patterns are exhibited by each of the V morphotype strains, as the growth conditions are varied. Some representative patterns are shown in Figure 7. Each branch is produced by a leading droplet of cells and emits side branches, each with its own leading droplet.

Microscopic observations revealed that each leading droplet consists of hundreds to millions of cells that circle a common center (hence the term vortex) at a cell speed of about 10 [[micro]meter]/s. Both the size of a vortex and the speed of the cells can vary according to the growth conditions and the location of the specific vortex in the colony [ILLUSTRATION FOR FIGURE 7 OMITTED]. Within a given colony, both clockwise and anticlockwise rotating vortices are observed. The vortices in a colony can also consist of either a single or multiple layers of cells. We occasionally observed vortices with an empty core, which we refer to as "bagel" shaped. After formation, the number of cells in the vortex increases, the vortex expands, and it translocates as a unit. The speed of the vortices is slower than the speed of the individual cells circulating around its center.

Bacterial cells are also contained in the trails left behind the leading vortices. Some are immobile, while others move, swirling with complex dynamics. The migrating groups of cells are reminiscent of the "worm" motion of

Cooperative organization of bacterial colonies: from genotype to morphotype.

slime mold or schools of multicellular organisms. The whole intricate dynamics is confined to the trail of the leading vortex, and neither a single cell nor a group of moving cells can pass out of the boundary of the trail. Only vortices formed in the trails can break out of the trail and create a new branch.

The microscopic observations also revealed that the bacterial motion is performed in a fluid on the agar surface. As is the case of the other morphotypes, this wetting fluid is also assumed to be excreted by the cells and/or extracted by the cells from the agar (12). We did not observe tumbling motion nor movement forward and backward. Rather, the motion was exclusively forward along the long axis of the cell. Moreover, the cells tended to move in the same direction and with the same speed as the surrounding neighboring cells, in what appeared to be a synchronized group movement. Electron microscope observations showed that the bacteria have flagella, which suggests that the motility is likely to be swarming.

Close inspection of these observations enabled the construction of a model for colonial development of swarming bacteria, which is also applicable for gliding bacteria. The model is inspired by the communicating walkers model (12) (see above). Here, the swarming cells are represented by swarmers. Each swarmer has a forward propulsion force. The balance of this force and friction forces tends to set the swarmer's speed to a specific value. In keeping with microscopic investigations, we also include velocity-velocity interactions, which tend to set the swarmer's velocity to the mean velocity of its neighbors (33, 69, 79, 82). In addition, we assume that the swarmers produce an extracellular "wetting" fluid, which they secrete during colonial growth. This extracellular slime also influences the bacterial motion. In the model, the swarmers can move only if the level of the wetting fluid is above a threshold value.

The above features, which are derived directly from the observations, are sufficient to describe the collective migration of bacteria. However, an additional feature has to be considered to explain the emergence of vortices. We propose the new feature to be a rotational chemotaxis, which differs from the chemotaxis normally employed by tumbling bacteria such as E. coli (24, 25, 37).

Rotational Chemotaxis and Vortex Formation

Swarming bacteria do not tumble, therefore they must employ a different method to perform chemotaxis. We propose that each individual cell modulates its propulsion force according to the local concentration of a chemotactic signaling material. In a group of cells, such a response creates a propulsion force gradient. Together with the velocity-velocity interaction, this imposes a torque or local vorticity on the average motion of the cells. Therefore, a swarmer moving at an angle to the chemical gradient is subjected to a torque that causes the swarmer to twist towards the direction of the local gradient of the chemoattractant.

We have shown that chemomodulation can indeed lead to the formation of stationary vortices (fixed in size and at a fixed location, [ILLUSTRATION FOR FIGURE 8A OMITTED]), rotating "bagels", and other elements. All these elements are of a length-scale comparable to or smaller than an individual branch of a colony. During colonial development, these elements are organized to form the observed global pattern.

Modeling the Cooperative Organization of Colonies

When modeling colony formation, we must take into account that bacterial cells in a colony do not move in a predetermined space, and their number and state of activity is not conserved. While the colony expands and changes its shape, cells reproduce, and frequently (in the case of V morphotype) also sporulate. To provide the means for reproduction, movement, and other metabolic processes, the cells consume nutrients from the environment.

As with the T bacteria, we represent the metabolic state of a swarmer by an "internal energy." When sufficient food is available, the internal energy increases until it reaches a threshold energy, and the swarmer divides in two. When a swarmer is starved for long periods, the internal energy drops to zero and the swarmer "freezes."

As was proposed previously, we assume that starved cells (swarmers for which the internal energy is zero or below a certain level) emit a diffusive compound at a fixed rate, and this chemical acts as a chemorepellent modulator. We assume that the repellent material decays slowly, so that its concentration is almost constant over distances comparable to the typical size of a vortex (long-range chemotaxis). This is in contrast with the attractant concentration, which is assumed to vary considerably within a vortex (short-range chemotaxis). Thus, although the functional form of the repulsion term is similar to the term for attraction, it has a very different effect on the bacterial motion. It affects each vortex as a single unit and provides a mechanism for regulating colonial structure during colonial development. On each vortex, the repulsion acts to push the vortex outward on a curved trajectory. In Figure 8, we show results of numerical simulations where both long-range repulsive and short-range attractive

Cooperative organization of bacterial colonies: from genotype to morphotype.

chemotactic signaling terms are included. We also included in the simulations cell differentiation, i.e. a finite probability per unit time (about 1%/div) of the swarmers to become immobile. The immobile swarmers at the trail have a similar probability to regain mobility. This was motivated by microscopic observations revealing the presence of cells left behind the advancing vortices.

MYXOBACTERIA

The myxobacteria provide one of the most complex and fascinating examples of social behavior in the microbial world. This gram-negative motile organism employs coordinated gliding motility and exhibits two distinct life cycles, one involving vegetative growth under conditions of nutrient excess, and the other, sporulation and fruiting-body formation under conditions of nutrient starvation. Both vegetative growth and freiting-body formation involve a complex spectrum of communal behavior, including cooperative feeding, group motility, cellular aggregation and cohesion, and collective movement. In Myxococcus xanthus, two sets of genes, termed A (Adventurous) and S (Social), are involved in vegetative growth and are characterized by mutant phenotypes. Mutants in the A genes are termed S motile, and grow vegetatively only when the cells are present in sufficient number and only within one cell length of each other. Most of the mutants in this class sporulate. In contrast, S-mutants grow as individual cells, but cannot aggregate, and do not form spores or fruiting bodies (46, 47, 57). Remarkably, two different S mutants were found to form ramified branched tip-splitting patterns almost identical to the patterns associated with the T morphotype shown in Figure 2. Although M. xanthus lacks a mechanism for swimming and tumbling, it does respond to both chemoattractants and repellents (57). Moreover, it does contain a full complement of cherootaxis genes (termed frz) completely analogous to those present in E. coli and S. typhimurium. Frz proteins are thought to be involved in a signal transduction pathway early in cellular aggregation that also involves a cell-associated C-factor. This same factor also functions later in fruiting body development (50, 52). Interestingly, one of the Frz proteins was unable to carry out negative chemotaxis away from a variety of repellents and apparently controls the reversal frequency of M. xanthus cells. The biological importance of frz function was dramatically demonstrated in an experiment in which wild-type M. xanthus cells penetrated a prey colony and remained there until all of the food source was digested, while frz mutants invariably abandoned the microcolony, leaving the food source behind, thereby demonstrating the importance of the frz system of signal transduction for the feeding behavior of M. xanthus.

CONCLUDING REMARKS

We have described the mode and dynamics of cooperative organization leading to complex pattern formation in bacteria. Employing concepts from studies of patterning in nonliving systems coupled with morphotype characterization, we introduced a generic modeling approach to study the pattern-formation processes. Emerging directly from the modeling is the introduction of new, experimentally testable concepts such as rotational cherootaxis or the interplay between self-generated short-range, long-range, and nutritional chemotactic forces. Clearly, many questions remain to be unraveled. For example, given the enormous biodiversity in the microbial world, the question of the variety, distribution, and prevalence of morphotypes in various natural environments presents a formidable challenge to the microbial ecologist. Moreover, in our view, major new insights will be forthcoming as we begin to develop an understanding of the genetic basis of cooperative morphotype behavior. Furthermore, it is not unlikely that different strains with similar morphotype characteristics may in fact use alternative physiological and genetic strategies to generate the same type of pattern. Will the generic models developed here be applicable in such circumstances? It is clear that both pattern formation and morphotype transition require a mode of organized cellular communication and cooperative multicellular behavior (72), demonstrating once again that, under specific conditions, certain microbial populations can become transformed from a herd of individual cells to a community of cooperators with mutual interests.

ACKNOWLEDGMENTS

We gratefully acknowledge the contributions of Marianna Tcherpikov and Natalia Goldin as well as the excellent technical assistance of Ina Brainiss and Rina Avigad. Work in our laboratories was sponsored in part by grants from the Israel Science Foundation and US-Israel Binational Science Foundation.

Literature Cited

1. Adler J. 1969. Chemoreceptors in bacteria. Science 166:1588-97

2. Avetisov VA, Goldanskii VI, Kuzmin VV. 1991. Handedness, origin of life and evolution. Phys. Today 44(7):33-41

Cooperative organization of bacterial colonies: from genotype to morphotype.

3. Beck G. 1995. Isolation and characterization of Salmonella typhinurium genes involved in pattern formation. MSc thesis. Weizmann Inst. of Science, Rehovoth, Israel

4. Beck BJ. Downs DM. 1998. The apbE gene encodes a lipoprotein involved in thiamine synthesis in Salmonella typhimurium. J. Bacteriol. 180:885-91

5. Ben-Jacob E. 1993. From snowflake formation to the growth of bacterial colonies, part I: diffusive patterning in non-living systems. Contemp. Phys. 34:247-73

6. Ben-Jacob E. 1997. From snowflake formation to the growth of bacterial colonies, part II: cooperative formation of complex colonial patterns. Contemp. Phys. 38: 205-41

7. Ben-Jacob E, Cohen I. 1997. Cooperative formation of bacterial patterns. In Bacteria As Multicellular Organisms, ed. JA Shapiro, M Dwarkin, pp. 394-416. New York: Oxford Univ. Press

8. Ben-Jacob E, Garik P. 1990. The formation of patterns in non-equilibrium growth. Nature 343:523-30

9. Ben-Jacob E, Garik P, Muller T, Grier D. 1988. Characterization of morphology transitions in diffusion-controlled systems. Phys. Rev. A 38:1370-80

10. Ben-Jacob E, Shmueli H, Schochet O, Tenenbaum A. 1992. Adaptive self-organization during growth of bacterial colonies. Physica A 187:378-424

11. Ben-Jacob E, Schochet O, Tenenbaum A. 1994. Bakterien schliessen sich zu bizarren formationen zusammen. In Muster des Ledendigen: Faszination inher Entstehung und Simulation, ed. A Deutsch, pp. 109-26. Cologne: Vieweg Verlag

12. Ben-Jacob E, Schochet O, Tenenbaum A, Cohen I, Czirok A, Vicsek T 1994. Generic modelling of cooperative growth patterns in bacterial colonies. Nature 368:46-49

13. Ben-Jacob E, Schochet O, Tenenbaum A, Cohen I, Czirok A, Vicsek T. 1994. Communication, regulation and control during complex patterning of bacterial colonies. Fractals 2(1):15-44

14. Ben-Jacob E, Tenenbaum A, Schochet O, Avidan O. 1994. Holotransformations of bacterial colonies and genome cybernetics. Physica A 202:1-47

15. Ben-Jacob E, Cohen t, Schochet O, Aranson I, Levine H, Tsimiring L. 1995. Complex bacterial patterns. Nature 373:566-67

16. Ben-Jacob E, Cohen I, Schochet O, Czirok A, Vicsek T. 1995. Cooperative formation of chiral patterns during growth of bacterial colonies. Phys. Rev. Lett. 75(15):2899-902

17. Ben-Jacob E, Schochet O, Cohen I, Tenenbaum A, Czirok A, Vicsek T. 1995. Cooperative strategies in formation of complex bacterial patterns. Fractals 3:849-68

18. Ben-Jacob E, Schochet O, Tenenbaum A, Avidan O. 1995. Evolution of complexity during growth of bacterial colonies. In Spatio-Temporal Patterns in Nonequilibrium Complex Systems, ed. PE Cladis, P Palffy-Muhoray, pp. 619-34. Reading, MA: Addison-Wesley

19. Ben-Jacob E, Cohen I, Czirok A. 1997. Smart bacterial colonies. In Physics of Biological Systems: From Molecules to Species, ed. H Flyvbjerg, J Hertz, OG Mouritsen, K Sneppen, pp. 307-24. Berlin: Springer

20. Ben-Jacob E, Schochet O, Tenenbaum A, Cohen I, Czirok A, Vicsek T. 1996. Response of bacterial colonies to imposed anisotropy. Phys. Rev. E 53:1835-45

21. Ben-Jacob E, Cohen I, Czirok A, Vicsek T, Gutnick DL. 1997. Chemomodulation of cellular movement and collective formation of vortices by swarming bacteria and colonial development. Physica A 238: 181-97

22. Berg HC. 1993. Random Walks in Biology. Princeton, NJ: Princeton Univ. Press

23. Berg HC, Purcell EM. 1977. Physics of chemoreception. Biophys. J. 20:193-219

24. Berg HC, Tedesco PM. 1975. Transient response to chemotactic stimuli in Escherichia coli. Proc. Natl. Acad. Sci. USA 72(8):3235-39

25. Bischoff DS, Ordal GW. 1992. Bacillus subtilis chemotaxis: a deviation from the Escherichia coli paradigm. Mol. Microbiol. 6:23-28

26. Blair DE 1995. How bacteria sense and swim. Annu. Rev. Microbiol. 49:489-522

27. Blat Y, Eisenbach M. 1995. Tar-dependent and -independent pattern formation by Salmonella typhimurium. J. Bacteriol. 177(7):1683-91

28. Blat Y, Eisenbach M. 1996. Oligomerization of the phosphatase CheZ upon interaction with the

Cooperative organization of bacterial colonies: from genotype to morphotype.

phosphorylated form of CheY. The signal protein of bacterial chemotaxis. J. Biol. Chem. 271:1226-31

29. Bruno WJ. 1992. CNLS Newsl. 82:1-10

30. Budrene EO, Berg HC. 1991. Complex patterns formed by motile cells of Escherichia coil. Nature 349:630-33

31. Budrene EO, Berg HC. 1995. Dynamics of formation of symmetrical patterns by chemotactic bacteria. Nature 376:49-53

32. Cohen I, Czirok A, Ben-Jacob E. 1996. Chemotactic-based adaptive self organization during colonial development. Physica A 233:678-98

33. Czirok A, Ben-Jacob E, Cohen I, Vicsek T. 1996. Formation of complex bacterial colonies via self-generated vortices. Phys. Rev. E 54:1791-801

34. Devreotes E 1989. Dictyostelium discoideum: a model system for cell-cell interactions in development. Science 245: 1054-58

35. Deleted in proof

36. Deleted in proof

37. Eisenbach M. 1990. Functions of the flagellar modes of rotation in bacterial motility and chemotaxis. Mol. Microbiol. 4(2):161-67

38. Eisenbach M. 1996. Control of bacterial choemtaxis. Mol. Microbiol. 20:903-10

39. Ford WW. 1916. Studies on aerobic spore-bearing non-phatogenic bacteria, part 2: miscellaneous cultures. J. Bacteriol. 1:518-26

40. Fujikawa H, Matsushita M. 1989. Fractal growth of Bacillus subtilis on agar plates. J. Phys. Soc. Jpn. 58:3875-78

41. Fuqua WC, Winans SC, Greenberg EP. 1994. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. J. Bacteriol. 176:269-75

42. Fuqua C, Winans SC, Greenberg EP. 1996. Census and consensus in bacterial ecosystems: the LuxR-LuxI family of quorum-sensing transcriptional regulators. Annu. Rev. Microbiol. 50:727-51

43. Garrity LF, Ordal GW. 1995. Chemotaxis in Bacillus subtilis: how bacteria monitor environmental signals. Pharmacol. Ther. 68:87-104

44. Harshey RM. 1994. Bees aren't the only ones: swarming in gram-negative bacteria. Mol. Microbiol. 13:389-94

45. Hegstrom RA, Kondepudi DK. 1990. The handedness of the universe. Sci. Am. 262: 108-15

46. Hodgkin J, Kaiser D. 1979. Genetics of gliding motility in Myxococcus xanthus (Myxobacterales): genes controlling movement of single cells. Mol. Gen. Genet. 171:167-76

47. Hodgkin J, Kaiser D. 1979. Genetics of gliding motility in Myxococcus xanthus (Myxobacterales): two gene systems control movement. Mol. Gen. Genet. 171: 177-91

48. Kaiser D, Losick R. 1993. How and why bacteria talk to each other. Cell 73:873-87

49. Kessler DA, Koplik J, Levine H. 1988. Pattern selection in fingered growth phenomena. Adv. Phys. 37:255-87

50. Kim SK, Kaiser D. 1992. Control of cell density and pattern by intercellular signaling in Myxococcus development. Annu. Rev. Microbiol. 46:117-39

51. Kitsunezaki S. 1997. Interface dynamics for bacterial colony formation. J. Phys. Soc. Jpn. 66:1544-50

52. Kuspa A, Kroos L, Kaiser D. 1986. Intercellular signalling is required for developmental gene expression in Myxococcus xanthus. Dev. Biol. 117:267-76

53. Lackiie JM, ed. 1986. Biology of the Chemotactic Response. Cambridge, UK: Cambridge Univ. Press

54. Langer JS. 1989. Dendrites, viscous fingering, and the theory of pattern formation. Science 243:1150-54

55. Latifi A, Winson MK, Foglino M, Bycroft BW, Stewart GS, et al. 1995. Multiple homologues of LuxR and LuxI control expression of virulence determinants and secondary metabolites through quorum sensing in Pseudomonas aeruginosa PAO1. Mol. Microbiol. 17:333-43

56. Losick R, Kaiser D. 1997. Why and how bacteria communicate. Sci. Am. 276:68-73

57. MacNeil SD, Mouzeyan A, Hartzell PL. 1994. Genes

Cooperative organization of bacterial colonies: from genotype to morphotype.

required for both gliding motility and development in Myxococcus xanthus. Mol. Microbiol. 14:785-95

58. Matsushita M, Fujikawa H. 1990. Diffusion-limited growth in bacterial colony formation. Physica A 168:498-506

59. Matsushita M, Wakita J-I, Matsuyama T. 1995. Growth and morphological changes of bacteria colonies. In Spatio-Temporal Patterns in Nonequilibrium Complex Systems, ed. PE Cladis, P Palffy-Muhoray, pp. 609-18. Reading, MA: Addison-Wesley

60. Matsushita M, Wakita J, Itoh H, Rafols I, Matsuyama T, et al. 1998. Interface growth and pattern formation in bacterial colonies. Physica A 249:517-24

61. Matsuyama T, Matsushita M. 1993. Fractal morphogenesis by a bacterial cell population. Crit. Rev. Microbiol. 19:117-35

62. Matsuyama T, Kaneda K, Nakagawa Y, Isa K, Hara-Hotta H, Yano I. 1992. A novel extracellular cyclic lipopeptide which promotes flagellum-dependent and -independent spreading growth of Serratia marcescens. J. Bacteriol. 174:1769-76

63. Matsuyama T, Harshey RM, Matsushita M. 1993. Self-similar colony morphogenesis by bacteria as the experimental model of fractal growth by a cell population. Fractals 1(3):302-11

64. Mendelson NH. 1978. Helical Bacillus subtilis macrofibers: morphogenesis of a bacterial multicellular macroorganism. Proc. Natl. Acad. Sci. USA 75(5):2478-82

65. Mendelson NH. 1990. Bacterial macro-fibres: the morphogenesis of complex multicellular bacterial forms. Sci. Prog. 74:425-41

66. Mendelson NH, Keener SL. 1982. Clockwise and counterclockwise pinwheel colony morphologies of Bacillus subtilis are correlated with the helix hand of the strain. J. Bacteriol. 151(1):455-57

67. Mendelson NH, Salhi B. 1996. Patterns of reporter gene expression in the phase diagram of Bacillus subtilis colony forms. J. Bacteriol. 178:1980-89

68. Mendelson NH, Thwaites JJ. 1989. Cell wall mechanical properties as measured with bacterial thread made from Bacillus subtilis. J. Bacteriol. 171(2):1055-62

69. Reynolds CW. 1987. Flocks, herds, and schools: a distributed behavioral model. Comput. Graphics 21(4):25-34

70. Rodel W, Plaga W, Frank R, Knappe J. 1988. Primary stractures of Escherichia coli pyruvate formate-lyase and pyruvate-formate-lyase-activating enzyme deduced from the DNA nucleotide sequences. Eur. J. Biochem. 177:153-58

71. Schloss JV, van Dyk DE, Vasta JF, Kutny RM. 1985. Purification and properties of Salmonella typhimurium acetolactate synthase isoenzyme II from Escherichia coli HB 101/pDUC9. Biochemistry 24:4952-59

72. Shapiro JA. 1988. Bacteria as multicellular organisms. Sci. Am. 258(6):62-69

73. Shaw CH. 1991. Swimming against the tide: chemotaxis in Agrobacterium. Bio-Essays 13(1):25-29

74. Shimkets LJ, Dworkin M. 1997. Myxo-bacterial multicellularity. In Bacteria As Multicellular Organisms, ed. JA Shapiro, M Dwarkin, pp. 220-44. New York: Oxford Univ. Press

75. Smith RN, Clark FE. 1938. Motile colonies of Bacillus alvei and other bacteria. J. Bacteriol. 35:59-60

76. Stevens FS. 1974. Pattern in Nature. Boston: Little, Brown

77. Stock JB, Stock AM, Mottonen M. 1990. Signal transduction in bacteria. Nature 344:395-400

78. Stock J, Surette M, Park P. 1994. Chemo-sensing and signal transduction in bacteria. Curr. Opin. Neurobiol. 4:474-80

79. Toner J, Tu Y. 1995. Long-range order in a two-dimensional dynamical XY model: how birds fly together. Phys. Rev. Lett. 75(23):4326-29

80. Tsimiring L, Levine H, Aranson I, Ben-Jacob E, Cohen I, et al. 1995. Aggregation patterns in stressed bacteria. Phys. Rev. Lett. 75:1859-62

81. Turing AM. 1952. Philos. Trans. R. Soc. London Ser. B 237:37-72

81a. Van-Dyk TK, Ayers BL, Morgan RW, LaRossa RA. 1998. Constricted flux through the branched-chain amino acid biosynthetic enzyme acetolactate synthase triggers elevated expression of genes regulated by rpoS and internal acidification. J. Bacteriol. 180:785-92

Cooperative organization of bacterial colonies: from genotype to morphotype.

82. Vicsek T, Czirok A, Ben-Jacob E, Cohen I, Schochet O, Tenenbaum A. 1995. Novel type of phase transition in a system of self-driven particles. Phys. Rev. Lett. 75:1226-29

83. Wolf G, ed. 1968. Encyclopaedia Cinematographica. Gottingen: Institut fur Wissenschaftlichen Film

84. Wong KK, Suen KL, Kwan HS. 1989. Transcription of pfl is regulated by anaerobiosis, catabolite repression, pyruvate, and oxrA: pfi::Mu dA operon fusions of Salmonella typhimurium. J. Bacteriol. 171:4900-5

85. Woodward DE, Tyson R, Myerscough MR, Murray JD, Budrene EO, Berg HC. 1995. Spatio-temporal patterns generated by Salmonella typhimurium. Biophys. J. 68:2181-89