A Fractal Approach to Pattern Formation in Biological Systems

RADU DOBRESCU, LORETTA ICHIM, STEFAN MOCANU, STEFAN POPA
"Politehnica" University of Bucharest, Faculty of Control and Computers,
313 Splaiul Independentei, Bucharest
ROMANIA
radud@isis.pub.ro

Abstract: - The paper discusses the connection between pattern formation and nonlinear dynamics, focusing on the similarity between discrete patterns and fractal structures, and then describes different solutions to model reaction-diffusion systems as representative processes in morphogenesis. The option for a discrete model and the steps to design it as a fractal structure is argued. Construction of appropriate generic model is an important step towards understanding the bacteria. It is shown how a pattern with arbitrary complexity like a fractal pattern can be realized by a reaction-diffusion system. A specific example is the diffusion limited aggregation growth process, illustrated by the simulation of the evolution of a bacterial colony that shows the roles of instability and sensitivity in nonequilibrium pattern formation.

Key-Words: - morphogenesis, pattern formation, reaction-diffusion systems, fractal analysis, attractors, diffusion limited aggregation

1 Introduction

Bacterial colonies grown on agar plates assume a variety of sizes and shapes of often amazing beauty [1]. On harder plates, growth is limited by the diffusion of nutrients; colonies have fractal-like boundaries [2] and essentially expand by mass increase.

Such phenomena, in which microscopic organisms give rise to collective macroscopic behaviors, are interesting because of their multi-scale nature, but also because they involve live cells. They raise the intriguing question of universality [3] in the description of physical and biological [4, 5] pattern-forming systems and growth mechanisms. For instance, it was recently pointed out that constructal theory may be used to describe, in a unified fashion, the scaling properties of all forms of locomotion, biological as well as mechanical.

Understanding the growth and dynamics of bacterial colonies may also give clues on how multicellular structures could have arisen from unicellular organisms. At a more general level, bacterial systems provide examples of collective behaviors in ensembles of live beings. Such cooperative effects are observed at all scales in nature [6], from bacterial patterns to fruiting bodies made by myxobacteria, vortices in zooplankton, locust swarms, fish schools, bird flocks and animal herds. Regardless of their size, these phenomena may be modeled in terms of interacting, often self-driven, particles, which move freely in space or are constrained to the nodes of a lattice [7]. Ideas from classical physics are naturally transported to these models: the appearance of ordered, large-scale structures in an initially disordered system is analogous to a phase transition; macroscopic parameters such as density and local velocity field may be defined and their evolution described in terms of continuous models. Many models for the growth and dynamics of bacterial colonies, written at different scales, can be found in the published literature.

Our approach deals essentially with pattern formation in biological systems far from equilibrium state, trying to underline a connection between the general principles of morphogenesis, the dynamics of the reaction-diffusion systems and the fractal analysis as a tool for modeling such processes.

2 Pattern formation

2.1 Definitions and classifications

In recent times, reaction–diffusion systems have attracted much interest as a prototype model for pattern formation. The above-mentioned patterns (fronts, spirals, targets, hexagons, stripes and dissipative solitons) can be found in various types of reaction-diffusion systems in spite of large discrepancies e.g. in the local reaction terms. It has also been argued that reaction-diffusion processes are an essential basis for processes connected to morphogenesis in biology [8].

The variety of natural patterns makes it difficult to analyze and compare them in a systematic manner.

We address this problem by focusing on the computational aspects of pattern formation

processes. They are characterized in terms of the number of morphogenetic agents, the computing capability of each agent, and the forms of information transfer between the agents and their environment. This computational analysis can be applied to a wide range of patterns, but in this paper are considered only *discrete patterns*, which are structures, based on repetitive occurrences of predefined figures called *motifs*. A pattern is assumed to be invariant with respect to some isometries in the plane, called its symmetries. The set of all symmetries forms a group under composition. Fractal structures can be assimilated as discrete patterns.

Stevens distinguished four prototypical classes of patterns (see Fig.1: a) spirals, b) meanders, c) explosions, d) branching patterns) by considering different methods for connecting a set of regularly arranged points into a graph without cycles [9].

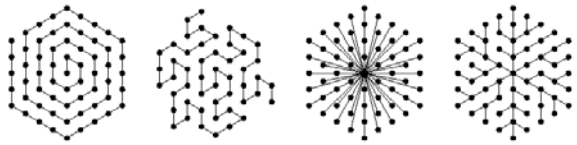

Fig. 1. Typical patterns proposed by Stevens

Patterns in each class were characterized by geometric attributes: uniformity, space filling, overall length of lines, and directness of lines. This characterization proved useful when analyzing natural patterns from the viewpoint of their optimality. Unfortunately, the optimality of the result does not offer a direct insight into the mechanisms that govern pattern formation. A classification that addressed this limitation was suggested by Bell, who distinguished the following three categories of branching patterns [10]:

- *Blind patterns*, in which branch initiation is controlled solely by the imposed program rules;
- *Sighted patterns*, in which the initiation of a new branch is influenced by factors detected by it in the immediate neighborhood;
- *Self-regulatory patterns*, in which branch initiation is controlled by the developing simulation itself, using communication via components of the existing framework, whether or not affected by environmental factors.

Focusing on the fundamental, algorithmic properties of pattern formation, the above classification makes possible to recognize and analyze similarities between apparently different realizations of similar patterns, and does not presuppose any computational framework for model construction. However, one of these models seems most suitable to simulate pattern formation. It is the model of a reaction-diffusion systems, which is in the same time the most appropriate to describe the construction of a fractal structure [11].

2.2 Bifurcations in pattern formation

After instability has produced a growing disturbance in a spatially uniform system, the crucial next step in the pattern-forming process must be some intrinsically nonlinear mechanism by which the system moves toward a new state. That state may resemble the unstable deformation of the original state. The system evolves in entirely new directions as determined by nonlinear dynamics. We now understand that it is here, in the nonlinearphase of the process, that the greatest scientific challenges arise. The inherent difficulty of the pattern-selection problem is a direct consequence of the underlying (linear or nonlinear) instabilities of the systems in which these phenomena occur. A system that is linearly unstable is one for which some response function diverges. This means that pattern-forming behavior is likely to be extremely sensitive to small perturbations or small changes in system parameters. Some important questions, therefore, are: Which perturbations and parameters are the sensitively controlling ones? What are the mechanisms by which those small effects govern the dynamics of pattern formation? What are the interrelations between physics at different length scales in pattern-forming systems?

Let now present a possible strategy for answering these questions. In dynamical systems theory, the stable steady solutions of the equations of motion are known as “stable fixed points” or “attractors”, and the set of points in the phase space from which trajectories flow to a given fixed point is its “basin of attraction”. As the control parameters are varied, the system typically passes through “bifurcations” in which a fixed point loses its stability and, at the same time, one or more new stable attractors appear. An especially simple example is the “pitchfork” bifurcation at which a stable fixed point representing a steady fluid flow, for example, gives rise to two symmetry-related fixed points describing cellular flows with opposite polarity. Many other types of bifurcation have been identified in simple models and also have been seen in experiments.

The theory of bifurcations in dynamical systems helps us understand why it is sometimes reasonable to describe a system with infinitely many degrees of freedom using only a finite (or even relatively small) number of dynamical variables. An important mathematical result known as the “center manifold theorem” [12] indicates that, when a bifurcation

occurs, the associated unstable trajectories typically move away from the originally stable fixed point only within a low-dimensional subspace of the full phase space. The subspace is "attracting" in the sense that trajectories starting elsewhere converge to it, so that the degrees of freedom outside the attracting subspace are effectively irrelevant. It is for this reason that we may need only a low-dimensional space of dynamical variables to describe some pattern-formation problems near their thresholds of instability — a remarkable physical result.

3 Classes of models for biological colonies

In the study of 2D patterns generated by propagating fronts in non-living systems, several modeling approaches to handle global morphology have been proposed. Stefan-like models [13] include an explicit boundary separating two regimes of diffusive fields. Phase-field-like models (Landau-Ginzburg models) [14] use only continuous fields to describe the system. The front in such models connects a stable phase to a meta-stable one, i.e. the growth term is bi-stable. In the limit of vanishing front width, the front can be replaced by an explicit boundary, and the model is reduced to a Stefan-like one [14]. Atomistic models, such as DLA [15], use particles moving stochastically (random walk) to describe molecules in a solution. In this approach solid matter is represented by stationary particles. DLA stands for diffusion limited aggregation, as particles from the solution aggregate to form the solid.

Preliminary attempts to model bacterial colonies were done by Ben-Jacob *et al.* [16] and Matsushita *et al.* [17]. While Matsushita *et al.* [17] attempted to measure growth parameters using Fisher-Kolmogorov equations, Ben-Jacob *et al.* [16] showed the limitation of this model. All the terms in this equation (diffusion term and a growth term which is unstable at zero density) agree with the microscopic bacterial details, but such models cannot produce the macroscopic branching patterns. Matsushita and Fujikawa [17] suggested a DLA model to describe the colony. This model can reproduce the global structure of the colony. However, the model has moving particles outside the aggregate, unlike the colony where the moving bacteria are inside the colony. Ben-Jacob *et al* [16] suggested that a phase-field-like model, with bi-stable growth term, can reproduce the global branching pattern. Bi-stable growth term does not agree with the details of the bacterial reproduction process, nor does it agree with the experiments (the growth process should be unstable near zero density).

It is well worth emphasizing the beneficial aspects of having a connection between a discrete model and a related continuum model. It is usually difficult to do much beyond simulation for a discrete model; so, having a continuum analog allows for analysis that helps guide the simulations and vice versa. For the DLA class of models, the relationship between these automata and the continuous approach to crystal growth as captured in the phase field model (and the free surface reduction thereof) has proven invaluable. Once the basics are understood, of course, one can modify the simulation to encompass more details of the actual system and thereby obtain more reliable results.

There is also a literature on using discrete models for other, more complex reaction diffusion processes; see for example the work by Kapral *et al.* [18] on simulations of 3D knotted labyrinths. In real reaction-diffusion processes, it is almost always the case that one is using the discrete model as a simpler stand-in for the true continuum dynamics; after all, one cannot hope to match the actual number of molecules (in the order of Avogadro number, 10^{23}) by discrete simulation entities, and the number of particles is enough such that a continuum description is valid (recall, though, the cutoff effect for type I systems). Using a small number of particles in the simulation as a stand-in introduces extra noise into the simulation, and this is the price one pays for a more flexible and more efficiently-coded numerical scheme. Similar remarks hold for lattice-gas automata [19], in which one uses discrete objects to model systems with fluid flow.

In biological multicellular systems, computational convenience is not the only reason why one can make good use of discrete entities. First, the numbers match more closely. The number of bacteria in a typical experiment is 10^9; one can almost approach these numbers computationally and therefore one is not plagued by the extra noise issue. Perhaps more importantly, cells contain large numbers of internal degrees of freedom which modulate their response to external signals form other cells. Hence, describing a population of cells with something as non-informative as a density field is usually insufficient. At the very least, one would have to introduce either new variables (which advect with the cell velocity as these are tied to the cells, and the Mean Orientation Field model) or even new coordinates [20], where the cell's age is taken as a relevant coordinate for the density field,

or the Orientation Dimension model where the cells' orientation is taken as a relevant coordinate for the density field); this makes for "ugly" continuum equations.
Tracking cells as individual objects makes it easy to add internal degrees of freedom; one just attach extra labels to the cell and postulate transition rules as to how these labels change in time. This flexibility is quite useful and hence many of the models to be discussed keep cells discrete. At the same time, though, continuum analysis is used to shed light on the simulations, and it forms an indispensable part of an integrated effort to understand microbiological pattern formation.

4 Modeling of reaction - diffusion systems

4.1 Basic paradigms

Qualitative studies of reaction diffusion systems of equations have probably begun from 1952. The first was shown by a mathematician, A. Turing, who is well known as a great pioneer in the field of computer science. He suggested by using a simple reaction-diffusion (RD) system a paradox that *diffusion enhances spatial in-homogeneity*, although we know, as common sense, that diffusion does enhance homogeneity in space (Turing, 1952) [21]. Turing's analysis simulated considerable theoretical research on mathematical models of pattern formation in chemical and biological systems, but Turing-type patterns were not observed in controlled laboratory experiments until 1990. He also claimed that such "diffusion-induced instability" gave the possibility to play a role in the mechanism of cell differentiation and morphogenesis arising in the field of developmental biology. The second was contributed by two neurophysiologists, A. L. Hodgkin and A. F. Huxley, who investigated the mechanism of impulses propagating along nerve fiber [22]. In the same year as Turing' paradox was stated, they proposed a model of nonlinear partial differential equations which is given by the coupling of a single RD equation with three ODEs in order to describe the propagation of impulses along the fiber. Their model could be numerically solved by using computer calculation. It is surprising that this model generates a traveling pulse wave with constant shape as well as velocity, in spite that it is described by diffusion equations. This indicates another paradoxical evidence of diffusion, that is, *suitable RD systems possibly generate a localized wave*.

Since 1952, RD systems of the form:

$$u_t = D\Delta u + F(u) \tag{1}$$

have been intensively investigated in the fields of not only applied science such as biology, chemistry, physics but also in mathematics. In a general case, equation (1) models the diffusion through a domain $\Omega \subset \Re^k$ of m interacting species or chemicals, where the i-th component u_i of $u = (u_1,\ldots, u_m)$ represents the density or concentration of the i-th reactants and $D = (d_1, \ldots, d_m)$ is the matrix of the diffusion constants $d_i > 0$ [23]. One hard mathematical task for the practical use of these models is to find appropriate vector supply terms F in such a way that the pattern formation process governed by the corresponding reaction-diffusion system coincides with the phenomenon observed in the laboratory experiments or in nature. Famous examples in this direction include the Kolmogorov-Fischer equations modeling the two-species interactions [24], the Field-Noyes equations modeling the Belousov-Zhabotinsky reactions in chemical kinetics [25], the Hodgkin-Huxley equations or the FitzHugh-Nagumo equations modeling the nerve impulse transmission [26].
Because the pattern formation process is the main subject in question, we can ask if the complexity of patterns modeled by the reaction-diffusion systems can be allowed to be arbitrary. A pattern is the eventual result of a time evolution of a biological or chemical or physical process and thus has the following two main features: a) Long-time effect and b) Great randomness of the initial conditions. Based on this observation, we see that a pattern is a kind of attractor. Here, by an attractor for a reaction-diffusion system we mean the mathematical object which attracts an open set of initial data in such a way that the trajectories starting from this initial data set eventually end up on the attractor in question (this is just the long-time effect of an attractor). The openness of the set of initial data guarantees the required great randomness of initial data which lead to the same pattern (attractor). Moreover, this openness corresponds to the practical need (e.g., for the computer simulation) that there is a positive probability that computed trajectories will tend to the attractor.
In the following we will give a method of constructing the vector supply term F for the purpose that the corresponding reaction-diffusion system has an attractor whose complexity is allowed to be arbitrary in some sense.

4.2 Steps in model construction

Let $n \in \aleph$ and $K \in \Re^n$ a given connected compact subset of arbitrary complexity. Then there exists a vector supply term F_K of the form:

$$F_K(\phi, v) = (A(\phi)v + f(\phi)) \quad (2)$$

for $\phi \in \Re$ and $v \in \Re^n$, where A is a smooth function on $\Re$ and f a smooth map on $\Re^n$, such that the corresponding reaction-diffusion system:

$$u_t = D\Delta u + F_k(u), x \in \Omega, t > 0 \quad (3)$$

of $n+1$ components $u=(\varphi,v)$, accompanying with the zero flux boundary condition, generates a dynamical system in the state space $C(\Omega)^{1+n}$ with the following properties:

(i) For each initial value $u_0 \in C(\Omega)^{1+n}$ the reaction-diffusion system (3) has a global unique solution u, $u(0) = u_0$, such that u is continuous in $\Omega x[0, \infty)$, and u_t, Δu as well as all partial derivatives are continuous in $\Omega x(0, \infty)$.

(ii) Each solution u of the reaction-diffusion system (3) starting from an initial value $u_0=(\varphi_0,v_0) \in C(\Omega)^{1+n}$ such that either $\varphi_0>0$ or $\varphi_0<0$ is asymptotically stable and converges to the set $\overline{K} = \{0\} \times K$ in the sense that $\omega(u,C) = \overline{K}$. As result, the connected compact set $\overline{K}$ is an attractor for the reaction-diffusion system (3) settled in the state space $C(\Omega)^{1+n}$.

Here we choose the zero flux boundary condition (i.e., the homogeneous Neumann boundary condition) and the positive initial condition, since they are probably the most interested boundary and initial conditions in the biological or chemical situation. Namely, the zero flux boundary condition reflects the self-organization mechanism of pattern while the positive initial condition restricts the pattern formation process to such a beginning circumstance that each of the reactants has a positive distribution all over the reaction domain. Thus, our statements should be considered as a partial but affirmative answer to the universality problem stated above and implies that any pattern (here $\overline{K}$) which is isomorphic to a connected compact subset (here K) of the Euclidean space $\Re^n$ can be seen as the final result of the pattern formation process governed by some appropriate reaction-diffusion system of $n + 1$ components. Moreover, it implies the following assertion: The make-up of a pattern $\overline{K} \cong K \subset \Re^n$ with arbitrary complexity (e.g., a fractal pattern) [27] can be realized by a reaction-diffusion system of the form (3) once the vector supply term F_K has been previously properly constructed.

Although the above construction is the product of theoretic thoughts, we were also interested in whether it is possible to derive such reaction-diffusion systems from any sequence of reasonable biochemical or physical situations, where one can consider that the first component φ of the vector $u=(\varphi,v)$ works as the activator and the rest components v as the inhibitor of the system). This mechanism simplicity throws light on the possibility of deriving reaction-diffusion systems of the form (3) from real world situations.

5 A dendritic growth pattern

5.1 General issues

The formation of patterns in the growth of bacterial colonies has extensively been studied experimentally. Resulting morphologies appear to depend on the growth conditions. They include well known morphologies such as Dense branched morphology (DBM) or Diffusion-limited aggregation (DLA), bunch much complex patterns and temporal behavior can be found.

One of these particular real world examples is the process of diffusion limited aggregation encountered in dendritic pattern formation [28]. The dendritic pattern formation process can be observed in different areas of nonequilibrium pattern formation: metallurgy (dendritic solidification), medicine (tumor growth), biology (bacterial colony development) and so on. In the most common situations, dendritic growth is controlled by diffusion—either the diffusion of latent heat away from the growing solidification front or the diffusion of chemical constituents toward and away from that front. These diffusion effects very often lead to shape instabilities; small bumps grow out into fingers because, like lightning rods, they concentrate the diffusive fluxes ahead of them and therefore grow out more rapidly than a flat surface. Similar situations occur in fluid dynamics, for example, in the “viscous fingering” problem [29]. The theory has been checked in numerical studies that have probed its nontrivial mathematical aspects [30]. The degree to which we can develop quantitative, predictive models of these phenomena will determine the degree to which we can control them and perhaps develop entirely new technologies.

5.2 Simulation of bacterial colonies growth

In 1989, a Japanese group [31] reported for the first time that bacterial colonies can grow elaborate branching patterns of the type familiar from the study of fractal formation in the process of diffusion limited aggregation (DLA). They showed explicitly that nutrient diffusion was the relevant dynamics responsible for the instability and can lead to different morphotypes [32].

So far, we have tested the models for they ability to reproduce microscopic dynamics of the bacterial colonies. All succeeded equally well, reproducing some aspects of the microscopic dynamics and the patterns in some range of nutrient level and agar concentration.

Each of the morphotypes exhibits its own profusion of patterns as the growth conditions are varied. The beautiful complex shapes obtained by simulation reflect sophisticated strategies employed by the bacteria for cooperative self-organization as they cope with unfavorable growth conditions.

The simulation model try to reflect the real behavior of cells observed through microscopy. Looking through the microscope at colonies of a certain *T* morphotype [33], one can see cells performing a random-walk-like movement in a fluid. We assume that this lubrication fluid is excreted by the cells and/or drawn by the cells from the agar culture medium. The cellular movement is confined to this fluid; isolated cells spotted on the agar surface do not move. A closer look at an individual branch (fig. 2) reveals a phenomenon of density variations within the branches. These 3-dimensional structures arise from accumulation of cells in layers. The aggregates can form spots and ridges which are scattered randomly, ordered in rows, or organized in a leaf-veins-like structure. The aggregates are not frozen; the cells in them are motile and the aggregates are dynamically maintained. The picture shows variation in the height of the branches. The more bacteria are in a unit area, the more layers the bacteria are in, and the higher the area seems. The boundary of the fluid thus defines a local boundary for the branch. Whenever the cells are active, the boundary propagates slowly as a result of the cellular movement pushing the envelope forward and production of additional wetting fluid. Electron microscope observations reveal that these bacteria have flagella for swimming.

The observations reveal also that the cells are active at the outer parts of the colony, while closer to the center the cells are stationary and some of them sporulate. Such spores are metabolically inert and exhibit a marked resistance to the lethal effects of heat, drying, freezing, deleterious chemicals, and radiation.

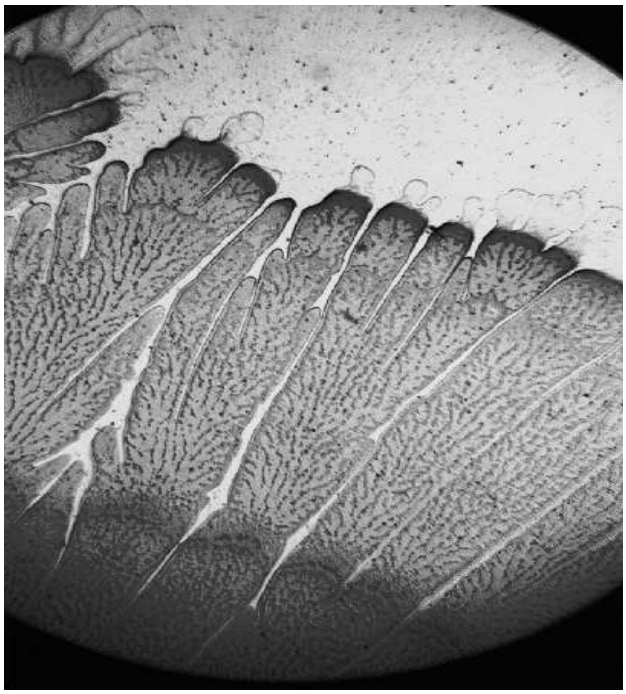

Fig. 2. Structure of ordered aggregates within branches.

6 Model implementation and results of simulation

6.1 Model design

We can gain much insight into instability mechanisms and nonlinear states from the continuous models of biological processes. Let us start with systems exhibiting diffusive instabilities. Initially, the simplest discrete analogue was afforded by diffusion-limited-aggregation (DLA). Here, discrete walkers move diffusively in space and attach to a growing cluster. In the limit of taking one walker at a time (i.e. of extremely slow growth) and purely irreversible attachment at any nearest-neighbor site, one obtains the classic DLA fractal [34]. It is well worth emphasizing the beneficial aspects of having a connection between a discrete simulation and a related continuum model. It is usually difficult to do much beyond simulation for a discrete model; so, having a continuum analogue allows for analysis that helps guide the simulations and vice versa.

The Discrete Walkers (DW) model describes the growth of colonies of *T* morphotype. The model was inspired by the diffusion-transition scheme proposed by Cohen in his Ph.D. thesis [33]. This scheme is a

hybridization of the "continuous" and "discrete" approaches used in the study of non-living systems. In the DW model, the bacterial cells are represented by discrete walkers that obey dynamic rules. The DW model also consists of at least one chemical field, namely nutrient concentration field, and additional element such as a free boundary of the colony. A walker in the DW model does not represent a single bacterium.

Each of the walkers will usually be taken to represent about one hundred cells. Each of the walkers has a position r_i and a metabolic state *Hi*. The lubrication fluid is not incorporated as such into the model, only its effects on the bacterial movement. The area occupied by the colony (wetted by the lubrication fluid) is defined by an on-lattice boundary representing the boundaries of the layer of lubrication fluid. To incorporate the swimming of the bacteria into the model, the walkers perform an off-lattice random walk within the area already occupied by the colony.

At each time-step each of the active walkers attempts to move from its location a step of size *d* at a random angle θ (θ chosen from $[0;2\pi]$ with uniform distribution), to a new location *r'* given by:

$$r' = r + d(\cos\theta;\sin\theta) \tag{4}$$

Although *d* is used in this equation as if it has length units, it's units are actually the square root of the units of a diffusion coefficient. These units compensate for fact that the number of steps of a walker per time unit is sensitive to the time-step of the model's simulation. If the units of *d* would have been length units, then the *effective diffusion coefficient* of the walkers in the bulk of the colony would have had been sensitive to the time-step of the model's simulation.

If the new location *r'* is outside the boundary, the walker does not perform that step, and a counter on the segment of the boundary which would have been crossed by the movement from *r* to *r'* is increased by one. When the segment counter reaches a pre-specified number of hits N_c, the boundary propagates one lattice step and an additional lattice cell is added to the area occupied by the colony. N_c is measured in units of length to the power of - *D* (where *D* is the spatial dimension of the simulation: 2 or 3) .The requirement of N_c hits represents the colony propagation through collective production of lubrication fluid and wetting of unoccupied areas. N_c is directly related to the food concentration, as more lubrication fluid has to be produced to push the boundary on a drier substrate.

We represent the metabolic state of the *i*-th walker by an "internal energy" *Hi*. The dynamics of this energy is given by:

$$\frac{dH_i}{dt} = cn_c - \frac{E_m}{\tau_r} \tag{5}$$

where *c* is a conversion factor from nutrient to internal energy and E_m represents the total energy loss for all processes (excluding reproduction) over the minimal time of reproduction τ_r. The nutrient consumption rate n_c is:

$$n_c = \min(\Omega_n, \Omega'_n) \tag{6}$$

where Ω_n is the maximal rate of nutrient consumption of a walker, and Ω'_n is the rate of nutrient consumption as limited by the local availability of nutrient. The maximal rate of nutrient consumption of a walker equals the consumption rate per cell times the number of cells represented by a single walker.

When sufficient nutrient is available, H_i increases until it reaches a threshold energy E_d and the walker divides into two. When the walker is "starved" for a long interval of time, H_i drops to zero and the walker "freezes". This "freezing" represents the transition into pre-spore state. For simplicity we have assumed in our experiments that the cellular density is suitable for sporulation, so that the limiting factor is the supply of nutrients.

The nutrients are represented by a field denoted *n*(*r; t*), and its dynamics is given by the diffusion equation:

$$\frac{\partial n}{\partial t} = D_n \nabla^2 n - \sigma_a n_c \tag{7}$$

where D_n is a diffusion coefficient and the last term on the RHS includes the consumption of nutrient by the active walkers whose density is denoted by σ_a,

$$\sigma_a \equiv \sum_{i\in(\text{active walkers})} \delta(x-r) \tag{8}$$

where $\delta(x)$ is the delta distribution function whose integral is the step function. The diffusion equation has zero-flux boundary conditions and uniform distribution of concentration n_0 as initial conditions.

6.2 Experimental results

The diffusion equation is solved on a triangular lattice with a lattice constant Δx, the same lattice on

which the boundary is outlined. For numerical stability the walkers' step length, $d\sqrt{\Delta t}$ (where Δt is the simulation's time-step), must be smaller than the lattice constant. All the simulations are stopped when the colony reaches a given radius.

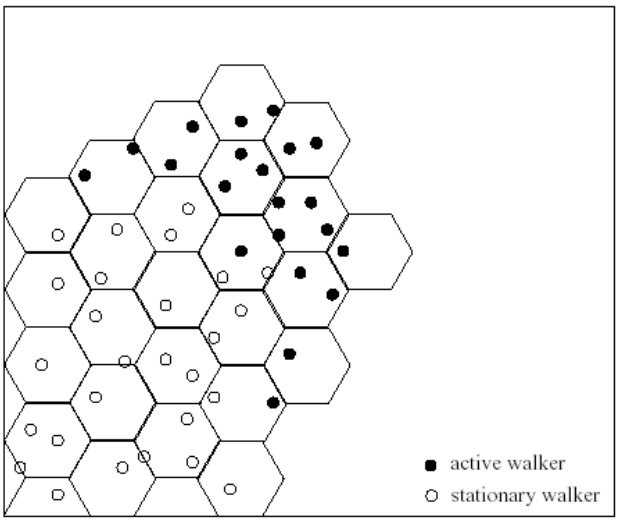

Fig. 3. A branch in a simulation of the DW model

The equation is solved on the tridiagonal lattice. The simulations are started with inoculum of walkers at the center and a uniform distribution of the nutrient.
Results of numerical simulations of the model are shown in fig. 3 (microscopic view: the hexagons are those lattice cells that were occupied by walkers and became part of the colony; the reaction-diffusion equations are solved on the whole lattice, weather part of the colony or not) and fig. 4 (colonial patterns, with N_c = 20 and the conversion factor c is 6, 8, 10 and 30 from left to right respectively).

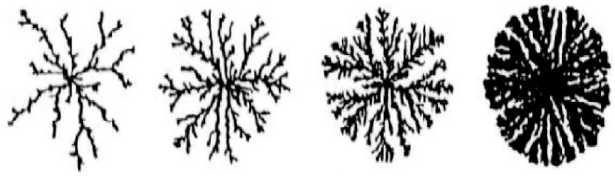

Fig. 4. Colonial patterns of the DW model

As in real bacterial colonies, the simulated patterns are compact at high nutrient concentration levels and become fractal with decreasing nutrient level. For a given nutrient level, the patterns are more ramified as the food concentration increases.
The results shown in figure 4 do capture some features of the experimentally observed patterns. However, at this stage the model does not account for some critical features, such as the ability of the bacteria to develop organized patterns at very low nutrient levels.

Fig. 5 shows the qualitative dependencies of the fractal dimension and the growth velocity of the nutrient concentration levels. As in the real bacterial colonies, in all the simulated colonies the growth velocity doesn't change significantly throughout the growth. The data are for typical runs of the DW model. The growth velocity is presented in arbitrary units.

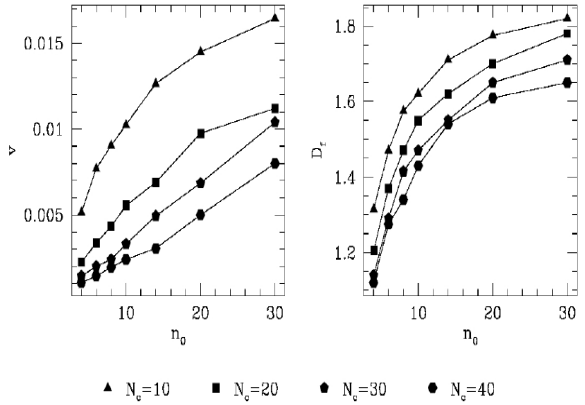

Fig. 5. Growth velocity (left) and Fractal dimension (right) as a function of initial food concentrations.

Clearly, the results are encouraging and do capture some features of the experimentally observed patterns. The branching patterns and the constant growth velocity are a manifestation of the diffusion field instability. From this perspective, it is quite reasonable that the effect of the instability is enhanced as the food concentration is raised and the motion of the bacteria is suppressed. This is analogous to lowering the diffusion coefficient of a bacterial density field (in a continuum description), which leads further into the diffusively unstable region of the parameter space.

7 Conclusions

We have considered morphogenesis as an inherently multilevel process, involving processes on different time and space scales and focused on the reciprocal influence between these levels. Thus, in our model morphogenesis is no longer a slave process, but unfolds by the interactions between pattern formation, the collective behavior of the cells, and its feedback to the pattern formation process.
We show here a pattern forming system, bacterial colony, whose discrete elements, the bacteria, are big enough to raise the question of modeling discrete systems. The DW model has explicit discrete units to represent the bacteria. The ratio between the walkers' size and the pattern's size is even bigger than the ratio in the bacterial colony.

Reaction-diffusion (RD) theory for pattern formation was considered in relation to processes of biological development. We have shown that RD-systems provide a strong framework for the modeling of growth processes and in particular, in biological systems. The RD-system model also permits the interaction of such systems in more complicated ways to provide emergent behaviors. This is particularly common in the development of multicellular organisms that served as models for simulation, because in addition to the feedbacks in the chemical dynamics, there is then another loop linking size and shape changes with the reaction-diffusion patterning of growth controllers in the growing region. We have found that regulation of shape change in particular ways (e.g. to make narrow-angle branching) demands new features in our chemical mechanisms.

The research presented here can be extended in several directions. One type of possible extension to the study presented here is to understand better the behavior of the biological systems, in order to choose the most adequate mathematical model. Another type of extension is to apply the approach of "generic modeling" to other types of biological systems. Yet another type of possible extension is the use of the same models (or closely related) in order to study various phenomena (not only biological) that are expressed in colonial pattern, and we have already started studies regarding the tumor growth.

ACKNOWLEDGEMENTS

This work was partially supported by the Romanian Ministry of Education and Research under Grant CNCSIS No. I 125/2007

References:

[1] Y. Yamazaki, Periodic growth of bacterial colonies *Physica D*, Vol.205, 2005, pp.136-53.

[2] H. Fujikawa, M. Matsushita, Bacterial fractal growth in the concentration field of nutrient. *J. Phys. Soc. Japan*, Vol.60, 1991, pp. 88-94.

[3] P. Ball, *The Self-made Tapestry: Pattern Formation in Nature*, New York Tokio: Oxford University Press, 1999.

[4] E. O. Budrene, H. C. Berg, Dynamics of formation of symmetrical patterns by chemotactic bacteria, *Nature*, Vol.376, 1995, pp. 49-53.

[5] E. Khain, L. M. Sander, Dynamics and pattern formation in invasive tumor growth, *Phys. Rev. Lett.*, Vol.96**,** 2006, 188103.

[6] J. K. Parrish, L. Edelstein-Keshet, Complexity, patterns, and evolutionary trade-offs in animal aggregation, *Science*, Vol.284, 1999, pp. 99-101.

[7] J. R. Raymond, M. R. Evans, Flocking regimes in a simple lattice model, *Phys. Rev. E*, Vol.73, 2006, 036112.

[8] L. G. Harrison, Kinetic *Theory of Living Pattern*, Cambridge University Press, 1993.

[9] P.S. Stevens, *Patterns in nature*, Little, Brown and Co., Boston, 1974.

[10] A. D. Bell, The simulation of branching patterns in modular organisms, *Philos. Trans. Royal Society London*, Ser. B, Vol.313, 1986, pp.143-169.

[11] P. Prusinkiewicz, Towards a computational theory of morphogenesis, In *Pattern Formation in Biology, Vision, and Dynamics*, 2000 pp. 91-95.

[12] J. Guckenheimer, P. Holmes, *Nonlinear Oscillations, Dynamical Systems, and Bifurcations of Vector Fields*, Springer, New York, 1983.

[13] E. Ben-Jacob, P. Garik, The formation of patterns in non-equilibrium growth, *Nature*, Vol.343, 1990, pp. 523-530.

[14] R. Kupferman, O. Shochet, E. Ben-Jacob, Z. Schuss, Studies of a phase-field model: Boundary layer, selected velocity and stability spectrum, *Phys. Rev. B*, Vol.49, 1992, 16045.

[15] L. M. Sander. Fractal growth processes. *Nature*, Vol.322, 1986, pp.789-793.

[16] E. Ben-Jacob, O. Shochet, A. Tenenbaum, I. Cohen, A. Czir´ok, T. Vicsek, Generic modeling of cooperative growth patterns in bacterial colonies, *Nature*, Vol.368, 1994, pp. 46-49.

[17] M. Matsushita, J. I. Wakita, T. Matsuyama, Growth and morphological changes of bacteria colonies, In P. E. Cladis, P. Palffy-Muhoray, editors, *Spatio - Temporal Patterns in Nonequilibrium Complex Systems*, Santa-Fe Institute studies in the sciences of complexity, Addison-Weseley Publishing, Company, 1995, pp. 609-618.

[18] A. Malevanets, R. Kapral, Links, knots, and knotted labyrinths in bistable systems, *Phys. Rev. Lett.*, Vol.77, 1996, pp. 767-770.

[19] J. P. Boon, D. Dab, R. Kapral, A. Lawniczak, Lattice gas automata for reactive systems. *Phys. Reports*, Vol.273, 1996, pp. 55-147.

[20] S. E. Esipov, J. A. Shapiro, Kinetic model of *Proteus Mirabilis* swarm colony development, *J. Math. Biol.*, Vol.36, 1998, pp. 249-268.

[21] A. Turing, The basis of morphogenesis, *Phil. Trans. Roy. Soc. London*, Vol.B237, 1952, pp. 37-72.

[22] A. L. Hodgkin, A. F. Huxley, A quantitative description of membrane current and its application to conduction and excitation in nerve, *J. Physiolog.*, Vol.117, 1952, pp.500-544.
[23] S. Z. Huang, Constructing Navier-Stokes equations with attractors of arbitrary complexity, *Physics Letters*, Vol.A282, 2001, pp.1-8.
[24] M. C. Cross, P. C. Hohenberg, Pattern formation outside of equilibrium, *Rev. Modern Phys*., Vol.65, 1993, pp. 851-1112.
[25] A. M. Zhabotinsky, A history of chemical oscillations and waves, *Chaos*, Vol.1, 1991, pp. 379-386.
[26] R. FitzHugh, Impulses and physiological states in theoretical models of nerve membrane, *Biophys. J.*, Vol.1, 1961, pp .445-466.
[27] R. Dobrescu, M. Dobrescu, St. Mocanu, Using self similarity to model network traffic, *WSEAS Transactions on Computers*, Vol.3, No.6, 2004, pp. 1752-1757.
[28] J. P. Gollub, J. S. Langer, Pattern formation in nonequilibrium physics, *Rev. Mod. Phys.*, Vol.71, No.2, 1999, pp.396-403.
[29] J. S. Langer, Dendrites, Viscous Fingers, and the Theory of Pattern Formation, *Science*, Vol.243, 1989, pp.1150-1156.
[30] A. Karma, W.-J. Rappel, Numerical Simulation of Three-Dimensional Dendritic Growth, *Phys. Rev. Lett.*, Vol.77, 1996, pp. 4050-4053.
[31] H. Fujikawa, M. Matsushita, Fractal growth of *Bacillus subtilis* on agar plates, *J. Phys. Soc. Jap.*, Vol.58, 1989, pp. 3875-3878.
[32] H. Fujikawa, M. Matsushita, Bacterial fractal growth in the concentration field of nutrient, *J. Phys. Soc. Jap.*, Vol.60, 1991, pp.88-94.
[33] I. Cohen, Mathematical Studies of Pattern Formation and Cooperative Organization in Bacterial Colonies, Ph.D thesis, Tel-Aviv University, 2004.
[34] T. A. Witten, L. M. Sander, Diffusion-limited aggregation, *Phys. Rev. B*, Vol.27, 1983, pp. 5686-5697.