

# Emergence of biodiversity in microbial populations

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## Abstract

It is well known that food stress resulting from density-dependent factors affects the mutation rate and hence promotes diversity. It is also known that in bacterial cultures the quality and composition of the growing media conditions the emergence of different spatial patterns. We would like to address the following question: how will the different spatial regions with well defined geometrical properties and density dependent stresses drive the emergence of diversity?

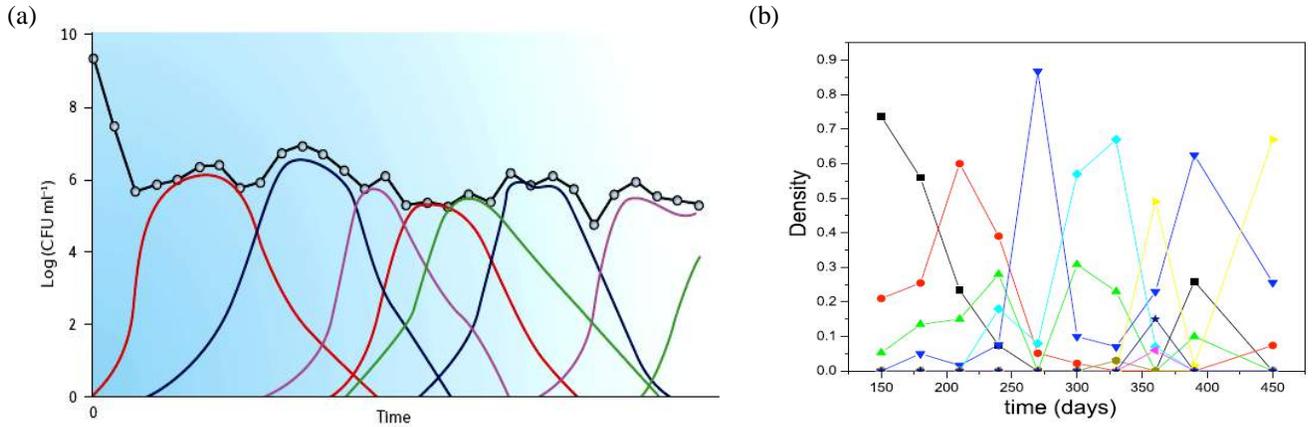
## Introduction

The process by which diversity is generated and maintained in ecosystems is at the core of ecological inquiry. And yet, it is striking that we do not have a simple and general quantitative theory of biodiversity generation and maintenance, notwithstanding several recent effort in this direction (1-5).

Bacterial population is a simple model to study biodiversity because bacteria are easily propagated, have large population size, have short generation time and are amenable to genetic analyses. Experimental results have shown that after long incubation period bacteria can undergo genetic polymorphisms in the form of mutant strains even in the absence of environmental heterogeneity (6-9). During this period it is possible to observe *periodic selection* in which population takeovers by more fit mutants associated with competitive exclusion (10) (Fig. 1a). *Escherichia coli* have been used to study bacterial evolution under these stress conditions (6, 12-14), experiments were conducted with prolonged periods of starvation using constant batch cultures; after the initial conditions, no further nutrients were added and no individuals were removed, the results showed that after periodic selection of mutants, mutants could not exclude each other (competitive equivalence) and therefore coexistence and diversity increased (Fig. 1b).

Stationary phase mutagenesis is also observed in *Bacillus subtilis* (16, 17). This bacterium has also shown the ability to generate distinct types of colony pattern (18). Depending on the substrate softness and nutrient concentration it can exhibit at least five types: diffusion-limited aggregation, compact Eden-like, dense branching morphology, concentric ring-like, and disk (19) (Fig. 2).

Building on this research, the question we would like to address is the following: how the different regions of the pattern in *B.subtilis*, with well defined geometrical properties (fractality, connectedness) and density dependent stresses (density, local density of food and bacteria) will drive the appearance of diversity and the evolution of different niches, which will in turn change the spatial pattern and the strategies to exploit the different niches? To achieve our goal we use cellular automata to model the bacterial behavior.



**Figure 1. Population dynamics of long-term stationary-phase cultures. (a)** There is a dynamic equilibrium between newly created growth advantage in stationary phase (GASP) mutants and less competitive cells. Each colored line represents a different GASP mutant that appears during long-term incubation. The black line represents the total population density (11). **(b)** Coexistence of different colony morphotypes, indicated by colors, during long-term incubation in a single culture (15).

## Methods

### *Model in one dimension*

To describe the population dynamics during long-term stationary-phase cultures we generated a model based on a cellular automaton in one dimension with periodic boundary conditions. The evolution rules are the following:

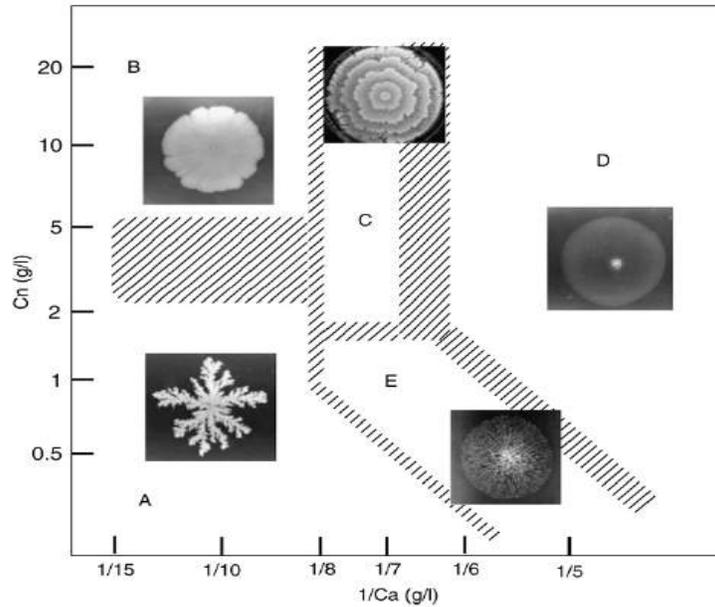
Each cell can be in  $n$  different states: State 0 if the cell is empty or a dead cell, state 1 if the cell is wild type and state 2- $n$  if the cell is a mutant. At time zero a seed colony is placed in the center of the vector. This model has one evolution time in which every  $F$  steps the cell can proliferate and every  $M$  steps the cell dies. At each evolution step a bacteria can mutate (with a normal probability distribution centered at zero and standard deviation as a parameter). If the value obtained is different from zero, this is subtracted from the value of  $F$  from the cell that generated it (e.g. if the mother cell has a  $F = 12$  and the value obtained from normal probability distribution is 1, the new cell has a  $F = 11$ ). When a cell proliferates it consumes resources  $= k / F$  where  $k$  is a constant; if the cell can not proliferate because of a dearth of resources, a new chance to mutate is possible. When a cell dies it gives  $k/10$  resource units.

The parameters of the model are: Number of cell (CN), fertility time ( $F$ ), mortality time ( $M$ ), number of total cycles (TC), standard deviation ( $\sigma$ ) and initial concentration of nutrients (values between  $N$  and  $1.3 \cdot N$ ).

### *Measure of diversity*

To determinate the diversity in the system we calculated the *evenness* value, which quantifies how even is the allocation of biomass among mutants. Evenness  $= \frac{H}{H_{max}}$  where

$$H = - \sum_{i=1}^n p_i \ln(p_i)$$



**Figure 2. Morphological diagram for *Bacillus subtilis*.** Region A corresponds to diffusion-limited aggregation, region B to compact Eden-like, region C concentric ring-like, region D to disk and region E to dense branching morphology (20).

### *Model in two dimensions*

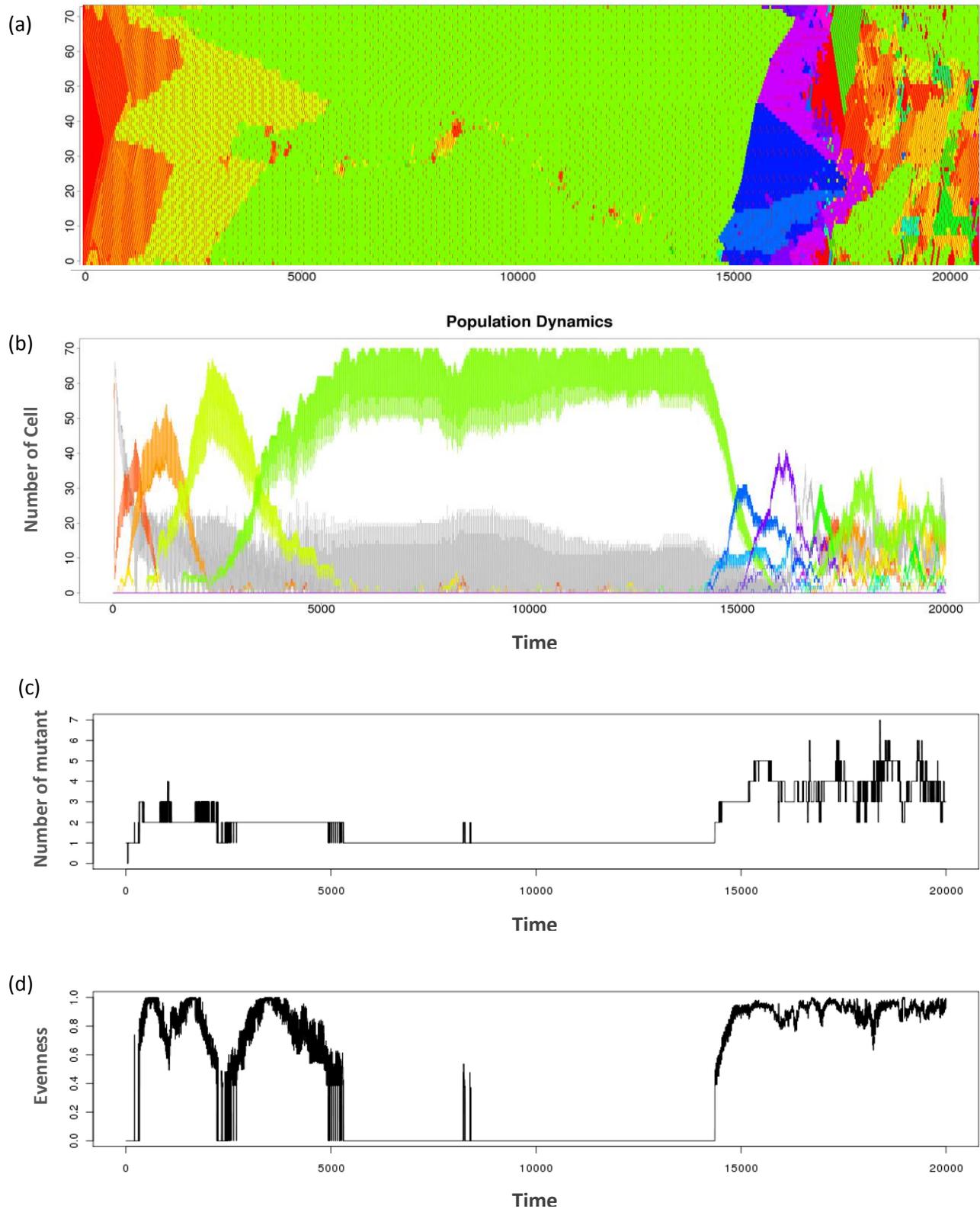
We chose dense branching morphology to describe the population dynamics in different regions of the pattern in *Bacillus subtilis*. Our model is based on cellular automaton in two dimensions with a regular hexagonal grid. To simulate the morphology we used several rules of the model proposed by Badoual *et al* (20) in which the evolution rules are:

Each cell can be in three possible states: State 1 if the bacterium can migrate, state 2 if the bacterium can proliferate and state 3 if it becomes inactive. There are two different times: proliferation ( $T_p$ ) and migration ( $T_m$ ).

At time zero a seed colony of bacteria is placed in the center of the grid and in each cell the available nutrient amount  $N$  is semi-uniform (each cell can have a value between  $N$  and  $1.3 \cdot N$ ). In the initial phase bacteria migrate with a radius proportional to the migration time. At the end of this phase, all seed bacteria are set to state 2.

The proliferation time is composed of two parts: In the first period  $T_p'$  the bacteria that can proliferate generate bacteria in state 2 while in the second period  $T_p''$  bacteria generated are in state 1. Each bacterium in state 2 places a new bacterium in the three free sites with the largest distance from the center. At the end of  $T_p$  the bacteria in state 2 become inactive (state 3). We considered the following proportion:  $T_p'/T_p'' = T_p/T_m$ .

During the migration time the bacteria that are in state 1 start migrating. They may move only to a free site. If this position is occupied, the bacterium does not move. Each migrating cells produce inactive cells in the place previously occupied. At the end of  $T_m$  the bacteria in state 1 change to state 2 with a probability proportional to  $T_p/T_m$ .



**Figure 3. Model of population dynamic.** (a) Simulation of growth evolution, each color is a different mutant, axis x is time and axis y is the space, the parameter are:  $N = 60000$ ,  $F = 18$ ,  $M = 37$ ,  $\sigma = 0.2$ ,  $CN = 70$ ,  $TC = 20000$ . (b) Population dynamics of distinct cells. (c) Number of distinct mutants in each step. (d) Evenness measure in each step.

Only bacteria in state 2 consume nutrients. To mimic nutrient's diffusion, we assume that at each time step a bacterium eats  $n$  nutrient units on its site,  $n - 1$  on its nearest neighboring sites and  $n - 2$  on the next-to-nearest neighboring sites.

Proliferating bacteria can only fill sites where the amount of nutrients is larger than  $n$  and bacteria on sites where the amount of nutrients is zero (or less) become inactive.

The rules are cyclic, i.e. at the end of a cycle (one proliferation phase and one migration phase), bacteria go back to state 2 and a new cycle starts. The number of cycles is denoted by  $T_f$ , but the bacteria can grow with a maximum radius equal to 180 (because the petri dish, in which bacteria grow, has a spatial limitation).

To simulate the long-term stationary-phase we considered the same rules of the model in one dimension in which every living cell have mortality time ( $M$ ), fertility time ( $F$ ). The cell can only proliferate in sites that have been previously occupied. Each time a mutant may arise with normal probability distribution (mean = 0, standard deviation = parameter). We allow each dead cell give 9 units of resource to cell in which it lived, 8 units to nearest neighboring sites and 7 to next-to-nearest neighboring sites. The final number of cells depends on the evolution of the model.

The parameters of this model are: Number of initial cell ( $IC$ ), migration time ( $T_m$ ), proliferation time ( $T_p$ ), mortality time ( $M$ ), fertility time ( $F$ ), number of cycles ( $T_f$ ), standard deviation ( $\sigma$ ) and initial concentration of nutrients ( $N$ ).

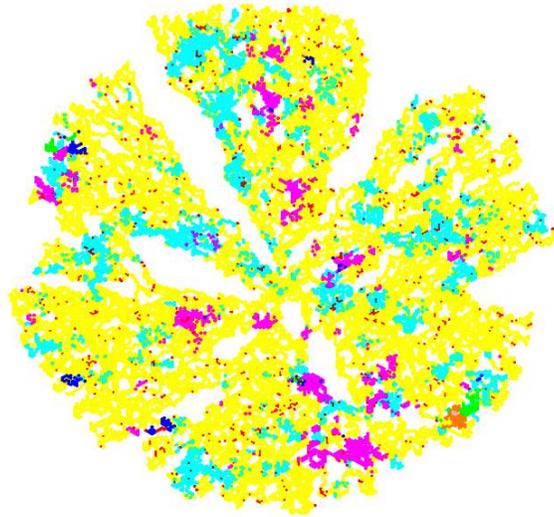
## Results

We have performed various simulations to find the most appropriate parameters of the model in one dimension that can reproduce the evolution of bacteria. We chose to start the simulation with  $N = 60000$ ,  $F = 18$ ,  $M = 37$ ,  $\sigma = 0.2$ ,  $CN = 70$ ,  $TC = 20000$  (Fig. 3a). The cells are, at the beginning, without scarce food (high value of  $N$ ), the values of  $M$  and  $F$  considered that the cell can reproduce at least 2 times per life cycle. The  $CN$  only alters the time when the cell is able to invade the whole system. The value of  $\sigma$  allows the emergence of a new mutant with a probability equal to 0.0124, slightly higher than the rate of spontaneous mutation (21).

To analyze the evolution of populations (different genotypes) we observed the number of each mutant for each time (Fig. 3b), in the first 5000 cycles is possible to observe how the different mutants, with a fertility time lower, invade almost all the system. For a long period only one mutant (green line) is invading all, it is possible because the new mutants could have shown a higher  $F$ . When the concentration of resources is low the mutation rate is higher, this allows the coexistence of different mutants, in which the fertility time is not an advantage to invade the whole system.

To determine the diversity of the system we calculated the time evolution of the number of different genotypes and the evenness value. Figure 3c and 3d shows that in the beginning there are between two and four distinct mutants, with varying evenness. By the end of the simulation, however, the number of different mutants is between four and seven with high and less variable evenness. This indicates that in the last period the diversity is higher in presence of more distinct mutants.

The result in two dimensions is represented in Fig. 4. The morphology obtained is very similar with the results of Badoual *et al* (20), here we considered the same values of  $T_p$ ,  $T_m$  y  $N$ . On the other hand is possible to observe that the distinct mutants, represented with different colors, invade local space as a result of spatial limitation of growth. This could happen because there is a spatial pattern in density



**Figure 4. Simulated pattern of bacteria with its different populations.** Yellow are wild type, red are dead cells and the other colors represent different mutants. The parameters are:  $T_p = 4$ ,  $T_m = 6$ ,  $T_f = 4500$ ,  $F = 7$ ,  $M = 15$ ,  $\sigma = 1$ ,  $N = 7$ . The standard deviation was only changed to observe the phenomenon at a less time.

dependent stresses that in turn are reflected in a spatial pattern in mutation and diversity, which may be linked to the evolution of different niches.

### **Conclusion and Future work**

Mutations are fundamental for the process of adaptation, innovation and diversity. In bacterial population the emergence of a new mutant with better fitness tends to replace other mutants through periodic selection. However, there is a point at which the bacteria, through different mechanisms, can coexist and biodiversity is allowed.

Our one-dimensional model reproduces this phenomenon, by considering only the variation in the rate of mutation that is dependent on resource concentrations in which different mutants can coexist independently of their fitness when nutrients are scarce. Figure 3c shows how after long periods of growth the number of different genotypes is higher than in an environment rich in resources. Complementary to this in the figure 3d it is possible to observe how the evenness value is constant and close to one, indicating that the distinct mutants have similar biomass (less variation between each group).

On the other hand, the model in two dimensions shows how the invasion of different genotypes is only local and the diversity is seen in a general context. The interesting thing about this is that applying the same rules for both models produce distinct results, indicating that the space limitation is essential to the evolution of the population.

For future work, it is important to do experiments to test for the spatial pattern of mutation, because there does not yet exist experimental evidence that shows the diverse emergence with spatial restrictions. Another important point is to be able to describe other types of patterns, including the influence of mutants on spatial pattern.

Finally, we could generate a model using more robust differential equations than the cellular automata.

## Acknowledgments

This work was funded by the Santa Fe Institute's International Program.

## References

1. **Hubbell, S.P** (1997). A unified theory of biogeography and relative species abundance and its application to tropical rainforests and coral reefs. *Coral reefs* 16: S9-S21.
2. **Hubbell, S.P** (2001). The unified neutral theory of biodiversity and biogeography. Princeton University Press, Princeton.
3. **Huisman, J., A.M. Johansson, E.O. Folmer and F.J. Weissing** (2001). Towards an solution of the plankton paradox: The importance of physiology and life history. *Ecology Letters* 4: 408-411.
4. **Allen, A.P., J.H. Brown and J.F. Gillooly** (2002). Global biodiversity, biochemical kinetics, and the energetic-equivalence rule. *Science* 297: 1545-1548.
5. **Brown, J. H., J.F. Gillooly, A.P. Allen, V.M. Savage and G.B. West** (2004). Toward a metabolic theory of ecology. *Ecology* 85:1771-1789.
6. **Zambrano, M.M., D.A. Siegele, M. Almirón, A. Tormo, and R. Kolter** (1993). Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. *Science* 259: 1757-1760.
7. **Rosenzweig, R.F., R.R. Sharp, D.S. Treves and J. Adams** (1994). Microbial evolution in a simple unstructured environment: Genetic differentiation in *Escherichia coli*. *Genetics* 137: 903-917.
8. **Riley, M.S., V.S. Cooper, R.E. Lenski, L.J. Forney and T.L. Marsh** (2001). Rapid phenotypic change and diversification of a soil bacterium during 1000 generations of experimental evolution. *Microbiology* 147: 995-1006.
9. **Maharjan, R., S. Seeto, L. Notley-McRobb and T. Ferenci** (2006). Clonal adaptive radiation in a constant environment. *Science* 313: 514-517.
10. **Atwood, K.C.L.K. Schneider and F.J. Ryan** (1951). Periodic Selection in *Escherichia coli*. *Proceedings of the National Academy of Sciences, (USA)* 37: 146-155.
11. **Finkel, S.E., E. Zinser and R. Kolter** (2000). Long-term survival and evolution in the stationary phase. Pp. 231-238 In: Storz, G. and R. Hengge-Aronis (eds) *Bacterial Stress Responses*. ASM Press, Washington D.C.
12. **Finkel, S.E. and R. Kolter** (1999). Evolution of microbial diversity during prolonged starvation. *Proceedings of the National Academy of Sciences (USA)* 96: 4023-4027.
13. **Zinzer, E.R. and R. Kolter** (1999). Mutations enhancing amino acid catabolism confer a growth advantage in stationary phase. *J. Bacteriol.* 181: 5800-5807.
14. **Zinzer, E.R. and R. Kolter** (2000). Prolonged stationary-phase incubation selects for lrp mutations in *Escherichia coli* K-12. *Journal of Bacteriology* 182: 4361-4365.
15. **Keymer, J. E., M.A Fuentes and P.A. Marquet**. Unpublished.
16. **Sung, H.M. and R.E. Yasbin** (2002). Adaptive, or Stationary-Phase, Mutagenesis, a Component of Bacterial Differentiation in *Bacillus subtilis*. *Journal of Bacteriology* 184: 5641-5653.

17. **Robleto, E. A., C. Ross, R. E. Yasbin, and M. Pedraza-Reyes** (2007). Stationary Phase Mutagenesis in *Bacillus subtilis*: A Paradigm to Study Genetic Diversity Programs in Cells Under Stress. *Critical Reviews in Biochemistry and Molecular Biology*. 42:327–339.
18. **Ohgiwari, M., M. Matsushita and T. Matsuyama** (1992). Morphological Changes in Growth Phenomena of Bacterial Colony Patterns. *Journal of the Physical Society of Japan*. 61: 816-822.
19. **Wakita, J., K. Komatsu, A. Nakahara, T. Matsuyama and M. Matsushita** (1993). Experimental Investigation on the Validity of Population Dynamics Approach to Bacterial Colony Formation. *Journal of the Physical Society of Japan*. 63: 1205-1211.
20. **Badoual, M., P. Derbez, M. Aubert and B. Grammaticos** (2009). Simulating the migration and growth patterns of *Bacillus subtilis*. *Physica A*. 388: 549-559.
21. **Drake, J. W., B. Charlesworth, D. Charlesworth and J. F. Crow** (1998) Rates of spontaneous mutation. *Genetics* 148: 1667–1686.