academicJournals

Vol. 7(26), pp. 3373-3378, 25 June, 2013
DOI: 10.5897/AJMR12.1266
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Computation of bacterial colonization using Monte Carlo simulations and scaling method

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Accepted 12 June, 2013

The formation mechanism of the growth of bacterial colony is studied by comparing the formation of the bacteria with the patterns obtained by Monte Carlo simulations using the diffusion limited aggregation algorithm. For this purpose, the morphological changes of the growing patterns are controlled by a sticking probability parameter, α, which represents the trajectories of the particles joining to the growing colonies, the complex reactions, and biological dynamics such as concentration of nutrient, temperature, and humidity in the growing environment. Specially, the sticking probability parameter is related to the biological activation and irreversible growth of the bacteria via growth energy for the mobility in the environment and perimeters of the colonies. Morphologies of the aggregation of the bacterial colonies have irregular, fractal, and compact structures. In this study, first, fractal dimensions are assessed for simulations and the real systems. The density of bacteria as ρ in region defined by circle of radius r centered at initial dropping seed from center through the perimeter is computed by using scaling method. Second, critical exponents of patterns are calculated. As a function of r, ρ reaches the asymptotic ρ_0 (α) following power-law $\bar{\rho} = \rho_0 + Ar^{-\gamma}$ with universal exponents γ = 0.47 for α = 1. The value of the main density for the bacterial patterns has $\rho_0 \sim \alpha^{-\beta}$, where β = 0.32 according to the scaling theory. Finally, the results obtained are found in good agreement with the experiments and can be useful for the researchers studying about bacterial colonization patterns.

Key words: Monte Carlo simulation, diffusion limited aggregation, sticking probability parameter, critical exponent, bacterial colony formation.

INTRODUCTION

The phenomena of bacterial colonization according to the substrate softness and nutrient concentration have received considerable attention for about 50 years ago. Particularly, colony pattern of bacteria species *Bacillus subtilis* (BS) has been vigorously studied from both experimental and theoretical viewpoints. Experimentally, bacterial strain is incubated on the agar plate's surface at the centre of a Petri dish with different concentration of nutrient, certain temperature, and certain humidity (Fujika and Matsushita, 1989). Another experimental study was performed on a morphological phase diagram of colonies of BS determined by varying both the concentration of nutrient and substrate softness (Wakita et al., 2001). They are both composed of five different patterns such as Diffusion Limited Aggregation (DLA)-like, Eden-like, Dense Branches Morphologies-like (DBM-like), concentric ring, and homogenous disk-like.

The structures of the colonies strongly depend on the dynamics of growth process such as nutrient concentration, temperature, and humidity. Many computer simulations have been carried out to investigate the relationship between the colony geometry

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and the formation mechanism (Li et al., 1995; Morikawa et al., 2003). They showed that patterns and colonies of bacteria formed through the DLA processes that have open and random branched structures with no natural length scale, so it can be categorized into fractals (Witten and Sander, 1983). The other models have been proposed to explain the variety of BS. DLA-like patterns have been interpreted as growth controlled by diffusion of nutrients in the context of DLA model. Ben-Jacob and co-workers proposed a 'communicating walkers model' to describe some parts of the morphological patterns of the bacterial colonization. This model reproduces the cross-over between different morphologies by coupling random walkers to fields representing the nutrients (Ben-Jacob, 1997). Other models were based on reaction-diffusion equations for bacterial density (Lacasta et al., 1999). But the scaling parameters for the bacterial patterns for both real and simulations were not determined in those models. It would also be useful to determine scaling parameters for the morphological changes of bacterial patterns using scaling method.

In this work, we determined the morphological changes of the bacterial colonization using Monte Carlo (MC) simulation. For this purpose, first, bacterial colony patterns with DLA algorithm were generated to find out the relationship between the variations in morphology using the Sticking Probability Parameter (SPP), α, for the concentration of the nutrient, temperature, and humidity. Then, fractal dimensions and critical exponents using scaling method were computed by the pattern obtained by simulation and compared with the experimental results from Fujika and Matsushita (1989). By using this relationship parameter to simulate the spatial and the temporal fluctuation, the biological significance and its relation to the formation of bacterial colonies observed in many real biological processes were discussed.

MATERIALS AND METHODS

Model and simulation

In this study, the MC simulation algorithm to determine morphological assessment was divided into two parts; one is the process of producing standard DLA patterns around the immobile incubated seed and the other is the DLA model that can be generalized by introducing a parameter called "SPP, α," for the representation of the conditions in lieu of the growth of the bacteria in a Petri dish (Witten and Sander, 1983). SPPs are used to model the course of the particles joining to the growing colonies and the complex reaction and biological dynamics on the growing environment such as concentration of nutrient, temperature, and humidity, respectively. Specially, SPP is related to the biological activation and irreversible growth of the bacteria via growth energy for the mobility in the environment and perimeters of the aggregate (Witten and Sander, 1983; Ben-Jacob, 1997). It allows us to vary both the morphological estimation and the fractal dimension D of the colony patterns. This is named as generalized DLA model for the azoic system. In the generalized DLA model, bacteria sticking to the colony on visiting active site in square lattice with sticking probability P:

$$P = \alpha^{3-B}$$

Where, α is some positive and adjustable parameter less than 1 ($0 < \alpha \leq 1$) and B is the number of nearest-neighbor occupied sites in the colony. For $\alpha = 1$, it generates DLA patterns as fractals. For smaller values of $\alpha \rightarrow 0$, it generates compact morphological patterns, $D \sim 2$ since active sites, $B = 3$ are more likely to get occupied than that of $B = 1$.

Numerical simulations are performed on a finite-size square lattice of $L \times L$ by Monte Carlo (MC) simulation. The length of the bacteria is chosen as linear dimension of $\varepsilon = 1$ lattice unit pixel. The occupied fraction as the bacterial density on the square lattice surface is given by:

$$\rho_0 = N L^{-d}$$

Where, N is the total number of bacteria on the square lattice surface, L is linear dimension of the square lattice, and d=2 is Euclidian Dimension, respectively.

RESULTS

It is simulated here as a generalized DLA model on a 2D square lattice used with the "SPP" α, which settled randomly down in square lattice surface. Comparing the patterns, one can see that it is statistically indistinguishable for the patterns. Linear dimension for the finite size square lattice in simulations is chosen to be $10^3 \times 10^3$ pixels. We generated about 150 colonies for the initial simulation parameters. Typical growth patterns are indicated in Figure 1A, B, C, and D for α = 0.009, 0.03, 0.07, and 0.5, respectively. The number and thickness of the branches by simulation are extremely similar with the small number of particles compared with the former experimental studies (Fujika and Matsushita, 1989; Wakita et al., 2001). The scaling parameters of these simulation patterns are summarized in Table 1 (see the colony patterns 2, 4, and 7 which are congruent with Fujika and Matsushita, 1989 and Wakita et al., 2001). If it is applied on a much larger-scale simulation, the number of branches would be increased and, accordingly the branches would become relatively thinner around the seeds (Ben-Jacob, 1997). Figure 1D shows a pattern generated by standard DLA model according to the sticking parameter α = 0.5. The standard DLA model in the non-azoic systems obtain the well-known pattern with a fractal dimension of about 1.71 in the square lattice (Witten and Sander, 1983).

From the resulting patterns of aggregates, it is calculated as the quantitative parameters for those characterizing the fractals, and they are depicted in Figure 2. We consider the following parameters: (i) fractal dimension, D, which is determined by standard method through box-counting curves, (ii) the main density of the frozen region of the patterns and critical exponents for them; (iii) scaling pentameters.

The fractal dimensions are computed via box-counting method (Bayirli and Kockar, 2010). In their study, the

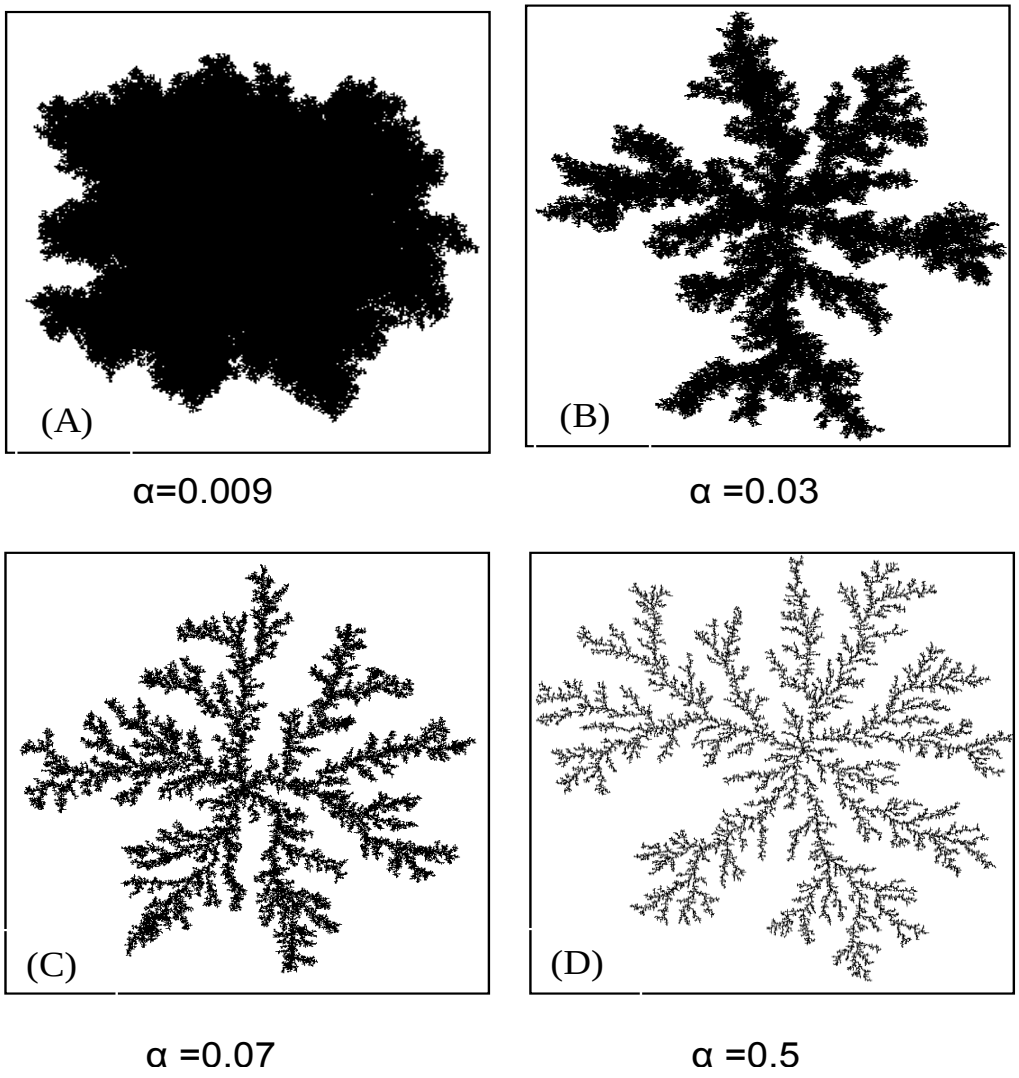

Figure 1. Typical morphological evaluations for bacterial pattern according to SPP α = 0.009, 0.03, 0.07 and 0.5 as concentration of nutrient, temperature and humidity.

Table 1. Values of the fractal dimension D, the SPP α, bacterial density ρ_0 and the critical exponent γ for both the simulation and the real bacterial colony aggregation patterns (Fujika and Matsushita, 1989).

Colony patterns	Fractal dimensions (D)	SPP (α)	Density (ρ_0)	Critical exponents (γ)
1	1.850 ± 0.018	0.009	0.827	0.012 ± 0.005
2	1.746 ± 0.012	0.030	0.764	0.423 ± 0.018
3	1.719 ± 0.004	0.070	0.572	0.443 ± 0.043
4	1.703 ± 0.006	0.090	0.504	0.417 ± 0.078
5	1.679 ± 0.004	0.100	0.326	0.433 ± 0.085
6	1.584 ± 0.023	0.500	0.278	0.464 ± 0.096
7	1.531 ± 0.026	1.000	0.185	0.477 ± 0.027
BS (Real)	1.711 ± 0.015	-	0.271	0.416 ± 0.014

box-counting method is explained and they compute the fractal dimensions of the manganese dendrite patterns growing on the surface of natural raw magnesite ore using this method. The box-counting dimension is

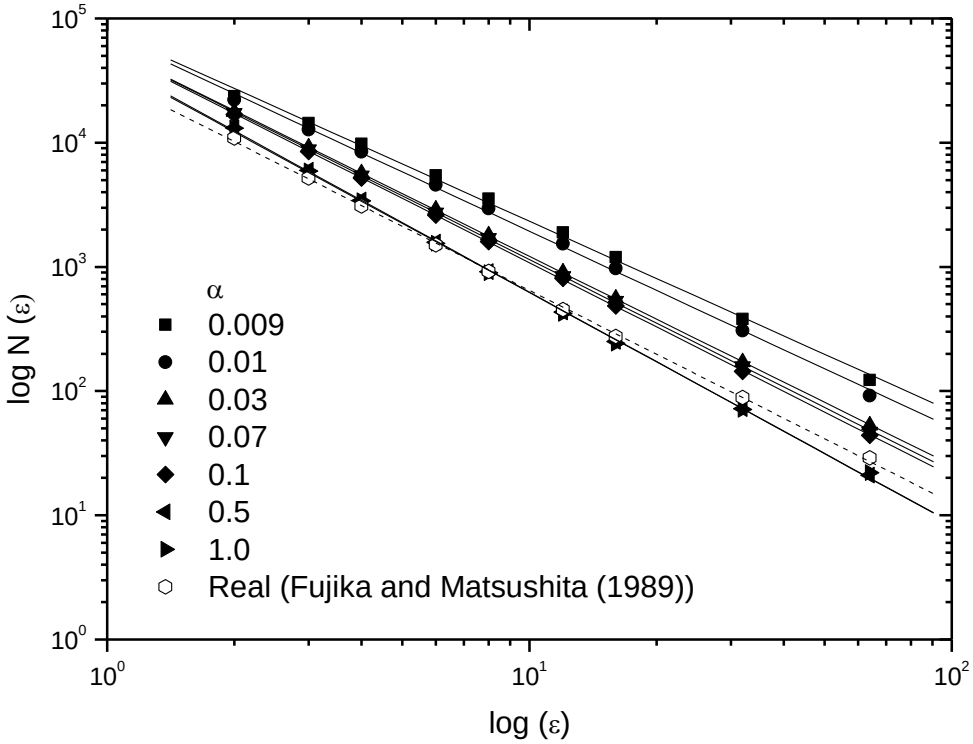

Figure 2. Fractal dimensions determined by box-counting method, for the different SPP. Seven line slopes between 1.531 and 1.85 are also plotted for comparison with experiments. If the SPP is 0.01 < α, the morphologies of the pattern have the Eden-like pattern. The colony of BS from experiment (Wakita et al., 2001) are computed as D = 1.711 ± 0.015 and as D =1.746 ± 0.012 at α = 0.03 from simulation patterns.

defined as:

$$D=\lim_{\varepsilon\to 0}\frac{\log N(\varepsilon)}{-\log(\frac{1}{\varepsilon})}$$

Where, N(ε) is the number of the box in the pattern.

In Figure 2, we show, in log-log plot, the number N of boxes of size ε that contain any part of pattern, versus the size of the boxes. The slopes of the lines represent the fractal dimensions. We observed that the cases that correspond to low nutrient have fractal dimension of about D = 1.531 with α = 1 and 1.746 with α = 0.03, showing good agreement with experiments, respectively. If the SPP is 0.01 < α, the morphologies of the pattern have the Eden-like pattern. Results are also summarized in Table 1. The value of the SPP α< 0.09 was counted as outlier for the critical exponent γ thus it was excluded from the analysis.

Ferreira et al. (2006) determined the morphological transition from DLA to ballistic aggregation using scaling hypothesis. It can be applied to morphological transitions for the colonies of bacteria that were obtained from simulations and can be compared to experimental patterns.

The morphological assessment of the BS colonies can be used for the scaling hypothesis. In order to quantify the morphological transition, the mean bacterial density in the inner regions of the colony can be assessed. The mean density $\bar{\rho}$ is defined as the ratio between the number of the occupied sites and the total number of sites in a region delimited by a circle of radius r centered at the initial seed. Since one expects asymptotically non-fractal patterns, the density must reach a finite value ρ_0 as $r\to\infty$. Nevertheless, the approach to the constant density is very slow and takes a scale in variant form:

$$\bar{\rho} = \rho_0 + Ar^{-\gamma}$$

Where, γ is the universal critical exponent as a correlation to the fractal dimension according to the scaling hypothesis and A is the constant. γ acts as an order parameter and a universal exponent for the morphological transition of bacterial patterns.

In Figure 3, Double logarithm plots of $\bar{\rho}$ - ρ_0 against r were fitted and its slope were obtained as α = 0.03. The linear fit of the data provided γ ≈ 0.45 for α = 0.03, and γ ≈ 0.42 for the experimental result from Fujika and Matsushita (1989).

In Table 1, the smallest value found was γ = 0.416 for

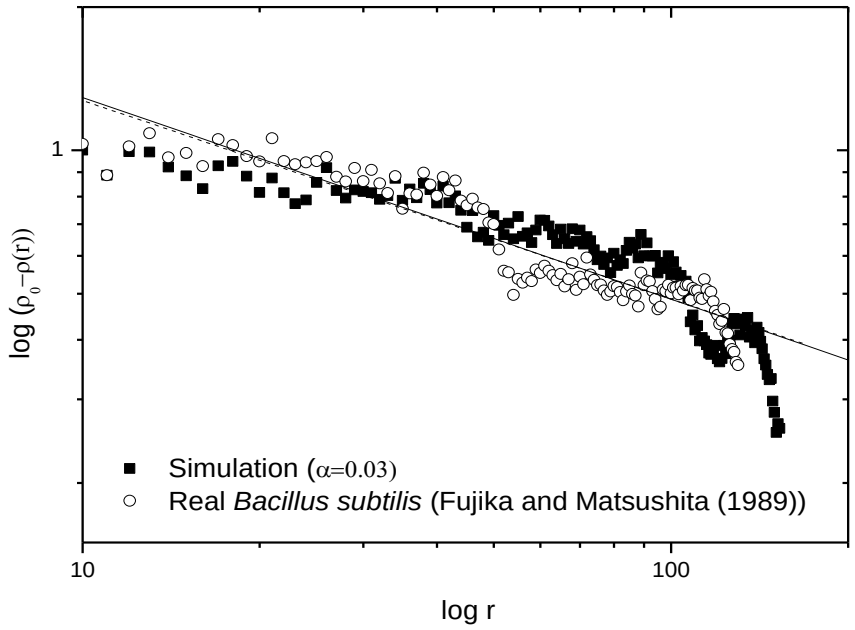

Figure 3. The double-algorithm plots of $\bar{\rho}$ (r) - ρ_0 as a function of r for distinct α = 0.03 values are shown according to DLA algorithm. The γ exponents for distinct values are presented in Table 1. The value of the critical exponent was computed as γ = 0.423 ± 0.018. One can observe that γ fluctuates from 0.423 to 0.477.

the real systems and the largest one was γ = 0.477 for the simulation respectively. This simulation suggests that the γ exponent and its value fluctuate about γ ≈ 0.439 ± 0.023 according to SPP. We also scanned the picture of the experimental result from Fujika and Matsushita (1989) and computed the mean bacterial density in the inner regions of the colony. Figure 3 shows both the simulation α = 0.03 for the value of SPP and the result of the experiment in Fujika and Matsushita's study. The densities were obtained by searching for the best linear fit in the larger linear region. To avoid the active region, we limited the fits to those data corresponding to half of the radial colony sizes.

Depending on α =1, 0.5, 0.1, 0.09, 0.07, 0.03 and 0.009 values, lattice with linear size $L = 10^3$ and with $N = 5.10^4$ particles, and 10 to 20 independent runs were used, respectively. One can observe a power law regime for 10 < r showing that the approaches to the stationary values obey the limit values. In Figure 4, the main density ρ_0 for the patterns is shown as a function of SPP α. ρ_0 acts as an order parameter, which changes at the critical values of the SPP α and they have the following relation:

$$\rho_0 \sim \alpha^{-\beta}$$

The universal exponent β for obtained slope of the data in Figure 4 was computed as 0.32(1) with variation from α = 1 to α = 0.009.

As the SPP α decreases, the main density ρ_0 increases. In α = 1, the pattern had a sparse branches in Figure 1D. The main density of bacteria is quite small. The surface of covered bacteria in a Petri dish was reduced and the pattern have compact image. The numbers of the numerical results in parenthesis represents the uncertainties.

The difference between these values is inside the error margins indicated in the parenthesis. The large uncertainties obtained in the exponents (5-10%) originated in the difficulty in the determination of the exact crossover points.

DISCUSSION

In the present work, the morphological growth of bacterial colony of BS was simulated using Monte Carlo simulations. Their scaling parameters for both the simulation and the real patterns were also computed. The patterns generated by standard DLA algorithm including the effects of biological conditions are concurred. Simply changing the SPP of the movement perpendicular to Monte Carlo movement incorporates the effects of concentration of nutrient, temperature, and humidity. Pattern formation in bacterial colony growth has mainly been explained by DLA growth mechanism. Furthermore, the fractal dimension of bacterial colony and the universal exponents at lower nutrient concentration was near that of a DLA pattern. The bacterial colonies can be compact and ring-structured in high nutrient concentration and humidity according to the experimental results of

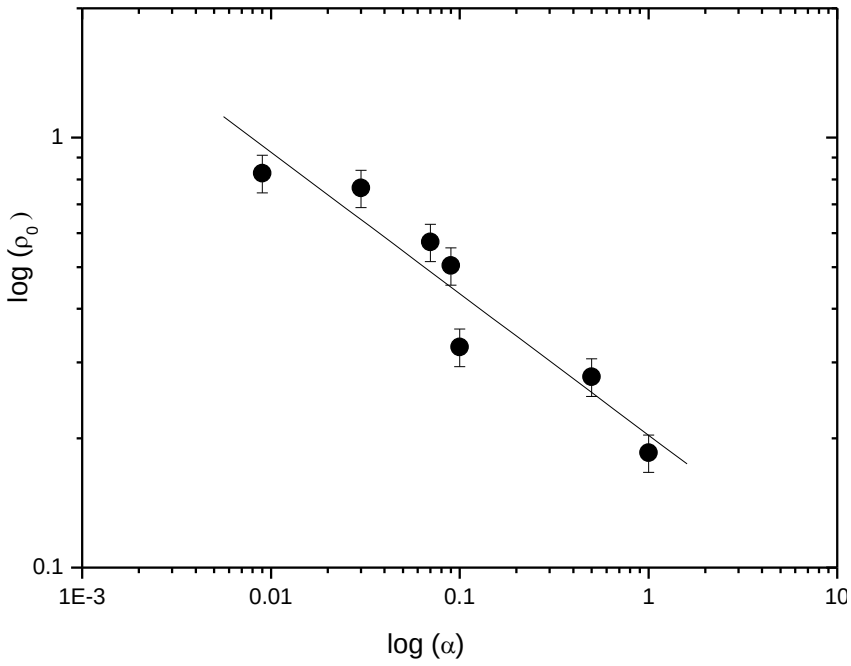

Figure 4. Double logarithm plots of ρ_0 against r for $0 < \alpha \leq 1$. The linear fit of the data provides $\beta \approx 0.32$.

Wakita et al. (2001). Despite the importance and simplicity of the DLA mechanism, there is still no rigorous theory of a convincing experimental demonstration to confirm that bacterial colony patterns actually belong to the DLA universal class. The results are in good agreement with the experiments. This model can explain bacterial colony patterns. It is a microscopic model and is found here that this model generates a wide variety of patterns under various conditions except for concentric ring-like patterns. Although size of bacteria relative to the overall pattern size in the experiment is smaller than that used in present computer simulation, growths of the obtained patterns can still be same as the observed real systems for the macroscopic scale. Thus, the small size difference between the simulation and the experimental results can be ignored. In conclusion, this microscopic model with respect to BS can also be applied to the other bacteria species such as *E. coli* or other microorganisms.

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