The Mechanism of Fractal-Like Structure Formation by Bacterial Populations

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Abstract. Three types of population growth and development of chemotactic motile bacteria *Escherichia coli* on semi-solid nutrient media are investigated: a) stable development – circular symmetrical waves; b) bursts; c) fractal-like self-organization. Experimental investigation of the burst formation is presented. The microscopic analysis of growing fractal-like structures is carried out, and a mechanism for such structure formation is suggested. It is supposed that fractal-like bacterial structures growth is based on the principle of successively forming multiple micro-bursts. A mathematical model has been suggested to reproduce the experimental results. The structures obtained by numerical modeling of population growth in the parameter space ‘substrate concentration – bacterial movement rate’ reproduce the corresponding experimental structures in the space ‘nutrient concentration in the media – the density of the media’.

Key words: Bacterial population, *Escherichia coli*, Fractal-like growth, Dynamics of spatial patterns, Mathematical model, Structure formation.

Growth and development of bacterial populations shows a great variety of geometrical shapes. Adler [1, 2] was the first to obtain bacterial population waves in the form of concentric circles (Figure 1a). A cooperative chemotactic response of bacteria to changes they induce in their medium was shown to be the reason for formation of such bacterial population waves. Experimental and theoretical investigations of the formation, propagation and interaction of the bacterial population waves have been considered in a number of papers [3–11]. Later some other forms of bacterial populations, such as grain-like structures [12–14], fractals [15–20] (Figure 1d,e,f) and bursts [21–23] were described in low agarized nutritive media (Figure 1b,c). E. Ben-Jacob *et al.* [15–18] were the first to reveal and investigate the formation of fractal-like structures in populations of the bacterium *Bacillus subtilis*. We find fractal growth of the same type in populations of the *E. coli* [19, 20], but the mechanism of the formation of such structures is not clear.

Here we investigate the relation between the process of burst formation and formation of the fractal-like structures in bacterial populations.

While studying the stability of bacterial waves [21–23] we have demonstrated that a cell burst might emerge and develop in front of the wave leading edge. Such bursts are caused by local fluctuations of the cell density at the growth front of the population. We inoculated bacteria *Escherichia coli*, strain C, at a point on the agar

Figure 1. Typical structures formed by growing populations of motile bacteria *E. coli* JM103 in a semisolid nutritive medium ('Serva' agar, glucose, M9): a) symmetric ring waves; b,c) bursts from symmetrically growing mother population; d,e) fractal-like structures; f) the fractal structure formed by the system of point-like bursts distinguishable by eye.

Table I. Average length of *E. coli* cells taken from: a) mother population; b) bursts immediately after their appearance; c) bursts 3 hours after their appearance

	Number of cells	Average cell length (μm)	Standard error
Experiment 1			
a	500	1.44	0.02
b	500	2.42	0.03
c	500	1.48	0.02
Experiment 2			
a	500	1.71	0.02
b	500	2.61	0.03
c	500	1.70	0.02

nutrient medium (0.3–0.4% w/v) containing peptone (1% w/v) and NaCl (0.5% w/v). The medium was poured into Petri dishes to a depth of about 3 mm. After inoculation, the Petri dishes were incubated at 37 °C. Formation of the bacterial rings started 4–6 hours after the inoculation, and then bursts were formed. We traced the growth dynamics of the bursts, taking samples during their development. Unlike during the stable ring propagation, the disturbance of population wave sta-

bility, seen as bursts, is characterized by a time dependent evolution of the average cell length. At the beginning of burst formation the maximum of the cell length distribution shifted towards greater lengths, from that of the mother population (1.44–1.71 μm) and becomes equal to 2.44–2.61 μm (Table I). After 3 hours the maximum of his distribution, as well as the average cell length, return to the initial values of 1.48–1.70 μm . The mechanism of such instability is the following: the burst is formed when cells located in the front of the expanding mother population, initially not numerous, considerably increase (due to division) their density before the overtaking front can incorporate them [21–23]. In some cases the bursts are separated from the mother population by demarcation zones of lowered concentration of substrates due to consumption by cells in the rear of the forming burst. Following the same principle bursts may emerge repeatedly (Figure 1c).

It should be noted that the bursts observed in various experiments differ considerably both in shape (ranging from compact, optically dense spots to dilute, loose structures) and in dimensions: from point-like, barely distinguishable by eye structures to those comparable in size to the original (mother) population.

It was found that such bursts also occur during fractal-like development of bacterial population. Microphotographs of various sites of the fractal-like structure formed by a developing population of *E. coli JM103* are given in Figure 2. It is seen, that at the periphery of the fractal structure bacteria form small clusters outside of the original mother population (Figure 2a). Such clusters are not observed closer to the central sites, where the growth is stopped (Figure 2b).

For various shapes of bacterial colony growth the following orders of bacterial wave propagation velocities are known [21]: symmetric waves – 4–9 mm hr^{-1} ; bursts – 0.5–1 mm hr^{-1} ; fractal grown – 0.1–2 mm hr^{-1} .

We suggest that, given the rather low propagation velocity of bacterial wave, fluctuations in the number of bacteria at the front would cause irregularities of its shape and produce bacterial clusters just in front of the growing fractal branch. In other words, the growth of fractal-like structure is the result of step by step, repeated formation of microbursts.

To check this assumption we have analyzed the propagation dynamics at various sites of the front of a growing fractal-like structure. *E. coli JM103* bacteria point-wise inoculated on the nutritive media containing ‘Serva’ agar –0.3%, glucose –1% and M9 medium, were incubated in a thermostat at 37 °C. After 10–15 hours dishes with an already formed fractal-like structure were examined under the microscope. The growth of a bacterial population front was recorded by a videorecorder every 10 min under constant temperature of 37 °C.

In such a way we recorded the growth dynamics of microbursts and the process of their merging with the mother population (Figure 3). It should be noted, that due to the small variations in humidity and lighting the structure growth under microscope was somewhere less than in control dishes that remained in the thermostat; however, the resulting deviation during 1 hour of the experiment did not exceed

Figure 2. Microphotographs of various sites of a fractal-like bacterial structure (*E. coli* JM103): in the center – general view, a – peripheral sites (multiple bursts are observed), b – central sites (no bursts are observed).

a few percents. The results of 5 experiments are given in Table II, and Figure 4 presents the front propagation velocities for the various cases.

The following results were obtained:

1. The maximum propagation velocity was of the mother population frontal sites with no microbursts observed in front of them, and ranged from 0.2 to 0.8 mm h⁻¹.
2. Propagation velocity of the mother population frontal sites with microbursts observed in front of them ranged from 0 to 0.4 mm h⁻¹ depending on the number

Figure 3. Growth dynamics of the front of a developing fractal-like structure (bacteria *E. coli* JM103).

Table II. Propagation velocities of the front of the growing fractal-like structure (bacteria *E. coli* JM103)

Number of experiment	Front velocity in the absence of burst (mm h^{-1})	Front velocity in the presence of burst (mm h^{-1})	Average rate of burst growth (mm h^{-1})
1	0.28	0–0.10	0.10
2	0.56	0.02–0.40	0.20
3	0.64	0.02–0.40	0.16
4	0.80	0–0.50	0.25
5	0.68	0.01–0.40	0.18

and vicinity of microbursts, i.e. in all cases a specific attenuation of the population growth was observed close to the microburst. The maximum distance at which the growth attenuation occurred was equal to 0.44 mm.

3. Growth velocity of microbursts ranged from 0 to 0.8 mm h^{-1} and greatly depended on the initial dimensions of a microburst: large well-shaped ones showed the maximum rate of growth; the growth of microbursts with a diameter less than $100 \mu\text{m}$ was not observable.

a)

b)

Figure 4. a) Propagation velocities of the mother population front sites with no distinguishable microbursts formed in front of them (bacteria *E. coli JM103*). b) Propagation velocities of the mother population front sites with observable microbursts formed in front of them.

4. In some cases we observed a slowing of the growth at separate sites of the mother population front edge, even though there were no microbursts visible in front of them.

Mathematical modelling.

The symmetric propagation and interaction of the bacterial population waves are well described by mathematical models based on the Keller-Segel equations [3, 4]:

$$\partial b / \partial t = g_b(S)b + \nabla(\mu(S)\nabla b - b\chi(S)\nabla S)$$

$$\partial S / \partial t = -g_s(S)b + D\nabla^2 S,$$

where b is the concentration of bacteria, S is the concentration of the culture substrate (which is an attractant for bacteria), $g_b(S)$ is the generation rate of cells, $g_s(S)$ – consumption rate of the substrate, χ is the chemotaxis coefficient, μ is the random motility coefficient analogous to Fickian diffusion coefficient, D is the diffusion constant of the substrate.

However, it is impossible to describe in terms of this model the asymmetric growth of a bacterial colony where, for example, microbursts or fractal development of a population take place. There emerges the problem of estimating the influence of the bacteria individual behavior on formation of asymmetric space structures. For this case E. Ben-Jacob et al. proposed the model [15] that included the following general features: (1) diffusion of nutrients; (2) movement of the bacteria; (3) reproduction and sporulation; (4) local communication.

We have modified the model proposed by E. Ben-Jacob. We trace movement of individual bacteria in the absolute system of coordinate, while changes of the substrate concentration is described by a differential equation, which is simulated by a $n * n$ grid with the grid step $dx * dy$.

The model may be characterized by the following main properties:

The differential equation describing the change of substrate $S(x, y, t)$ concentration is the following:

$$\frac{\partial S}{\partial t} = -\alpha g(s)b - \beta g(s)b_p + D_s \Delta S$$

where b is mobile bacteria, b_p is immobile bacteria, D_s is the substrate diffusion coefficient, $g(S) = S/(S + S_g)$, α, β, S_g are constants.

Bacterial cells are able to move both within each space cell or to transfer from one cell to another.

- at the initial time $t = 0$ N_0 of mobile bacteria are placed into the central cell containing nutritive medium;
- bacteria move either drifting freely straight forward or tumbling, with a random direction of movement after tumbling;
- the initial substrate concentration everywhere through the nutritive medium is S_0 ;

Figure 5. Dynamics of fractal-like structure formation in numerical experiments ($n = 80$, $S_0 = 0.5$, $N_0 = 100$, $V_b = 0.2$, $\alpha = 0.0075$, $\beta = 0.001$).

– the bacterial motility is restricted by some threshold concentration S_p of the substrate, i.e. if $S < S_p$ then the lowering of S is accompanied by the drop of the free drift period (τ) down to zero:

$$\tau(S) = \begin{cases} \tau_0 & \text{for } S \geq S_p \\ \tau_0 S/S_p & \text{for } S_z < S < S_p \\ 0 & \text{for } S < S_z \end{cases}$$

where τ_0 , and S_z are constants;

– the speed of a bacterium during the free drift is V_b , i.e. the length of the free drift is $L = V_b \tau(S)$;

– if a bacterium is in a space cell with the medium concentration $S < S_z$ where $S_z \ll S_p$, then it loses the ability to move;

The influence of the substrate concentration on the bacterial cell division is accounted for as follows:

1. If division of a cell located at coordinates (x, y) occurs at the time moment t_1 at the substrate concentration is $S \geq S_p$, then the next division of a bacterium should take place at the moment $t_2 = t_1 + T_b$, where T_b is the period of bacterial division;

2. In case when a cell is located in a region with the substrate concentration $S < S_p$, then the moment of next division is delayed: $t_2 = t_{02} + (S_p/S)dt$; where t_{02} is the previous value of t_2 ;

Figure 5 shows the dynamics of formation of fractal-like structures in numerical experiments with parameter values: $dt = 0.1$, $dx = dy = 1$, $S_p = 0.2$, $S_z = 0.07$,

Figure 6. Results of the natural experiments (bacteria *E. coli JM103*) with altering the agar density and peptone concentration in the nutritive medium.

$D_s = 0.25$, $\tau_0 = 2$, $T_b = 12$, $S_0 = 0.5$, $N_0 = 100$, $V_b = 0.2$, $\alpha = 0.0075$, $\beta = 0.001$.

The formation of fractal structures in real experiments (bacteria *E. coli JM103*) is influenced by the agar density and peptone concentration in the nutritive medium (Figure 6).

Figure 7. Results of the numeric experiments on model under variations of the Q and S_0 parameters ($Q = 1/V_b$).

1) Fractal growth has observed when agar concentration in the medium was not less than 0.3% and not greater than 0.5%, provided the concentration of peptone was not less than 0.4%.

2) With lowering the peptone concentration the fractal growth was suppressed and symmetric wave pictures were formed as: a) ring-like population waves with a strongly pronounced front or b) spots with diffuse borders.

For example, when the agar concentration was 0.45%, the fractal growth was suppressed in $\sim 20\%$ of the experiments. Complete suppression of the fractal growth was observed when the peptone concentration was decreased to 0.25% and lower. It is interesting that the combination of low peptone concentrations and relatively high agar concentrations ($C_a = 0.3\%$) showed the wave pictures which preserved the asymmetric character typical for fractal growth, but here no distinctive branching was observed.

3) When intermediate concentration values were used as well as fractal-like structures and ring-like population waves one could observe both the fractal-like structures and the population waves shaped as a circle or an arc.

In Figure 7 results of the numerical experiments with the mathematical model show the formation of various structures depending on the values of Q and S_0 , where $Q = 1/V_b$ corresponds to the concentration of agar in natural experiments, and S_0 corresponds to the pepton concentration C_p .

Fractal growth takes place when the V_b parameter values range from 0.2 to 0.5. With V_b equal to 0.3 the fractal growth is suppressed when $S_0 = 0.7$, at $V_b = 0.4$ the corresponding value is $S_0 = 0.5$, and at $V_b = 0.5$ ring structures are observed when $S_0 = 0.4$. It can be seen that the results of numerical experiments (Figure 7) agree with the experimental data given above.

Thus, the variety of structural metamorphoses in bacterial population waves can be explained both by general approach to their description and by considering individual behavior of bacteria, with only local interactions between them. The proposed model demonstrates the essential role of density fluctuations and cell motility in the development of fractal structures.

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