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EXPERIMENTAL ARTICLES

Bacterial Motion in Porous Media

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Abstract—The motion of chemotactically different *Escherichia coli* C600, cheB287, and AW405 cells was studied using a column packed with silica gel. The model chemotaxis of bacteria in porous media seems to be adequate for natural bacterial chemotaxis in soils. The porous structure of silica gel prevents interfering convective flows. Silica gel columns make it possible to separate bacterial cells differing in motility and chemotaxis. Relevant physical phenomena are discussed. The concept of fast and slow chemotaxis is considered.

Key words: bacterial chemotaxis, technogenic pollution, bioremediation, cell separation, biocontrol, soil, silica gel.

Severe human impact on the environment presents the problem of its bioremediation. One of the most efficient and ecologically sound methods of bioremediation is the use of microorganisms that are able to convert complex organic wastes to simple compounds utilizable by higher plants. The bioremediation of polluted soils is possible mainly due to the activity of soil bacteria, whose abundance in a particular soil is determined by its chemical composition, texture, climatic conditions in the region, and other factors.

The porous structure of soils does not hinder the swimming motion of bacterial cells, since soil pores have sizes ranging from several to hundreds of micrometers, which corresponds to the length of the straight-line segments of random bacterial motion [1, 2]. The absence of convective flows and the saturation of soil solution with mineral components and organic residues create ideal conditions for the natural motility and chemotaxis of bacterial cells. The close relationship between soil and its microflora probably results from the long evolutionary development of this symbiotic complex. In particular, the degrading capacity of soil bacteria and their cometabolic pathways have evolved in response to the close contact of bacterial cells with organic industrial wastes, which penetrate into the surrounding soil and adhere on the surface of soil microparticles. The study of the distribution and metabolism of soil bacterial populations in polluted soils should contribute to the development of ecologically safe methods of their remediation.

The motility and chemotaxis of degrading bacteria in liquid media have been quite well studied under in vitro laboratory conditions [3–5]. The kinetic model of bacterial motility and chemotaxis relates the locomotive parameters of particular cells and the whole bacterial population [6, 7]. In in situ experiments, this model should be supplemented by additional parameters that take into account the porosity of the medium saturated with nutrients. The simulation of bacterial behavior in porous media can provide insight into actual processes occurring in nature.

It is known that motile bacterial cells concentrate in a region where the concentration of chemical effectors is optimal [1, 2]. This phenomenon can be used for the separation of cells differing in motility and chemotaxis [8].

The aim of this work was to study the chemotactic behavior of three strains of *Escherichia coli* K-12 in a silica gel column through which a buffer solution was pumped. The porous structure of silica gel did not hinder the free swimming of bacterial cells in the liquid medium but prevented interfering convective flows.

MATERIALS AND METHODS

Experiments were carried out with three *Escherichia coli* K-12 strains: C600, cheB287, and AW405 (the two last strains were obtained from A.N. Glagolev, Department of Biophysics, Faculty of Physics, Moscow State University). Cells of strain C600 are not flagellated. Cells of strain cheB287 (met^-) have normal flagella but possess only tumbling motility because of low demethylase activity. Cells of strain AW405 (met^+) have normal flagella and possess normal motility [9].

Bacterial cells were grown at 35°C on a shaker (220 rpm) in nutrient broth to the exponential growth phase (OD = 0.3–0.5; (0.3–0.5) × 10^9 cells/ml), at which the motility and chemotaxis of cells are maximal. Cells were harvested and washed by centrifugation at 1000 *g* for 10 min and suspended in the so-called

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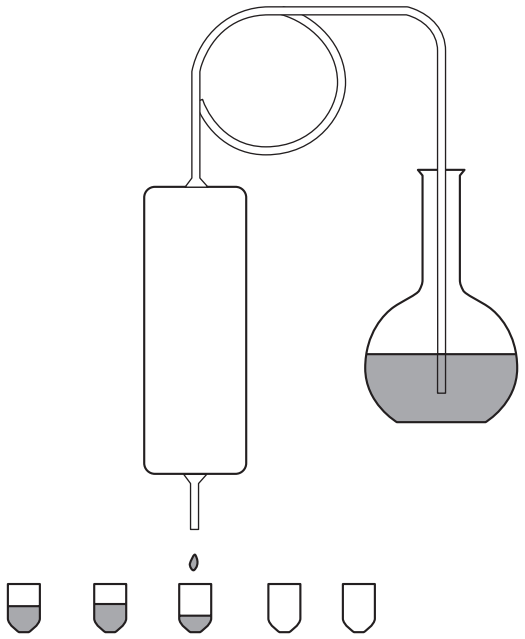

Fig. 1. Schematic representation of a silica gel column for studying bacterial chemotaxis in porous media.

CHE buffer (0.1 mM EDTA and 2 wt % glycerol in 10 mM potassium phosphate buffer with pH 7.0) [10, 11]. The same buffer was pumped through a column with silica gel granules 1 to 2 mm in diameter, which were preliminarily treated with hydrochloric acid and then neutralized at 140°C for 5 h.

Cells were enumerated by direct microscopic count in a Goryaev chamber and by plating onto minimal agar. In the case of the strain cheB287 (met^-), the agar was supplemented with methionine.

The silica gel column (length, 10 cm; volume, 20 ml; void volume, 8 ml) was equilibrated with CHE buffer, either with L-serine or without (Fig. 1). Bacterial suspension containing 2.8×10^8 cells/ml was applied onto the column in a volume of 1 ml and kept at room temperature (22–25°C) for a certain time *T*. Then the column was eluted with CHE buffer at a flow rate of 1 ml/min. The eluate was collected in 1-ml fractions, which were analyzed for cell concentration.

RESULTS AND DISCUSSION

Figures 2–4 show the distribution of bacterial cells among fractions eluted at $T = 0$ and $T = 2$ h. It can be seen that the distribution of C600 and cheB287 cells among the fractions did not change over the aforementioned period of time (2 h). In contrast, AW405 cells behaved as if they gradually sank and spread throughout the column. These data are in agreement with those of Aswad and Koshland, who showed that, unlike nonmotile bacterial cells, motile flagellated cells swimming in a steady liquid medium sink relatively quickly [11]. Generally, the behavior of cells in a bacterial population depends on chemotaxis (i.e., the directional motion of cells in a gradient of a chemoeffector) [11] and on gravitational and viscous forces [12].

To study the role of chemotaxis in the motion of bacterial cells in porous media, we performed the following experiment. The silica gel column equilibrated with CHE buffer was washed with 5 ml of the same buffer containing 10 mM L-serine (this amino acid is an attractant for AW405 cells) and then with 5 ml of the buffer without serine. This procedure gave rise to a serine gradient, whose smoothness was enhanced by the porous structure of the medium. Indeed, the flow of the buffer through the column produces whirls between silica gel granules. The whirls promote the mixing of the solutions with and without serine and thus enhance the diffusion of a sharp interface between these solutions. This process is favored by the diffusion of serine molecules into silica gel granules and the subsequent release of the molecules into the mobile phase. If required, silica gel granules impregnated with serine to specific concentrations can be packed into a column to produce a spatial gradient of serine of the necessary form.

After the serine gradient had been formed, 1 ml of a bacterial suspension was applied onto the column. Figures 4 and 5 show the distribution of AW405 cells among the fractions eluted from the silica gel column in the presence and absence of the serine gradient in the column. As is evident from these figures, AW405 cells, which are capable of chemotaxis, concentrate along the serine gradient. This was not the case with C600 and cheB287 cells, which are incapable of chemotaxis.

In the next experiment, we attempted to separate cheB287 and AW405 cells, which differ in their capability for motility and chemotaxis. The experimental procedure was the same as described above, with the exception that the suspension applied onto the column was a 1-ml mixture of 2.8×10^8 cells of each of the cheB287 and AW405 strains. As is evident from Fig. 6, the distribution patterns of cheB287 and AW405 cells in this experiment were similar to those observed in the above experiments with the monosuspensions. In other words, the statistical motion of a bacterial cell in a mixed suspension occurring in a chemical gradient does not depend on the presence of other cells in the suspension. The total pattern of bacterial motion in a population is the sum of the locomotive behaviors of particular groups of cells.

Comparative analysis showed that the number of viable cells passed through the column and determined by the method of plating onto agar media is about twofold less than the number of bacterial cells determined by directly counting them in a Goryaev chamber. This fact does not contradict the general tendencies observed in the distribution of different cells passed through the silica gel column, although the effect of silica gel on the viability of bacterial cells is of independent interest and requires further study.

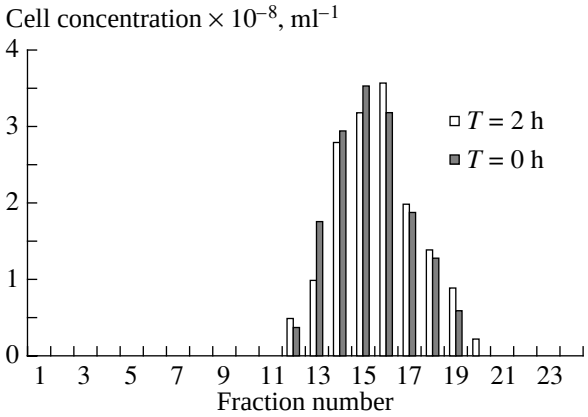

Fig. 2. Histogram showing the distribution of nonflagellated C600 cells among the fractions eluted from the silica gel column.

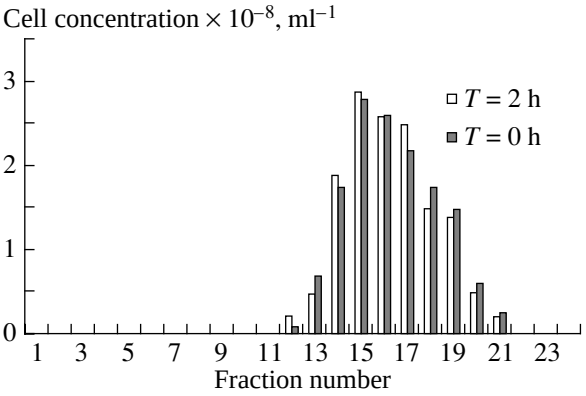

Fig. 3. Histogram showing the distribution of cheB287 cells among the fractions eluted from the silica gel column. The cells of strain cheB287 (met⁻) have normal flagella but possess only tumbling motility due to low demethylase activity.

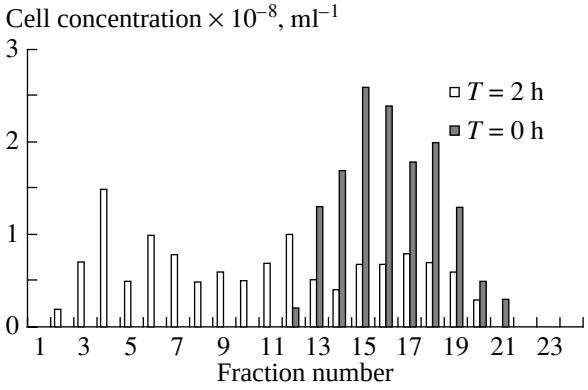

Fig. 4. Histogram showing the distribution of AW405 cells among the fractions eluted from the silica gel column without the attractant (L-serine) gradient. The cells of strain AW405 have normal flagella and possess normal motility.

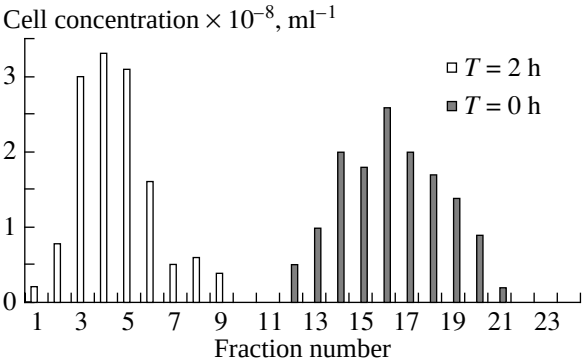

Fig. 5. Histogram showing the distribution of AW 405 cells among the fractions eluted from the silica gel column with the L-serine gradient.

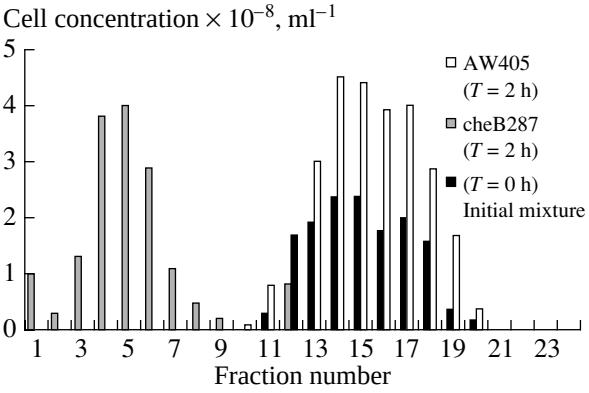

Fig. 6. Histogram showing the separation of AW405 and cheB287 cells in the L-serine gradient formed in the silica gel column.

The choice of silica gel for the last experiment was dictated by its simple chemical structure, affordability, and inertness to bacterial cells. Experiments with silica gel showed that this type of silicon dioxide is a suitable porous medium for the study of bacterial chemotaxis in aqueous media and for the simulation of the locomotive behavior of microbial populations in sandy soils. The experiments described in this paper show the promise of silica gel in separating cells and investigating the heterogeneity of microbial populations.

The described method of studying bacterial chemotaxis using silica gel columns differs from the other known relevant methods (the capillary method of Adler [10], the densitometric method [3], and the method of chemotactic rings on semiliquid agar [5]) in that it deals with large volumes of preparations (tens of milliliters against, for instance, several microliters in the Adler method) and great displacement distances of bacterial fronts (centimeters against millimeters in the conventional methods) covered in time $T = 2$ h. The question now arises as to whether such large distances are artifacts. Calculations, however, show that a swimming

cell has a linear velocity of about 20 μm/s and may cover a distance of about 14.4 cm in 2 h. Due to the random wandering of cells, their actual displacement in liquid media is considerably shorter. The situation considerably changes in the case of porous media, in which the velocity of bacterial fronts may reach several centimeters per hour. Such a velocity of bacterial fronts is in agreement with the theoretical parameters of bacterial chemotaxis [7]. The physical causes of fast bacterial chemotaxis in porous media require further investigations.

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