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EXPERIMENTAL ARTICLES

The Study of Bacterial Chemotaxis to Naphthalene

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Abstract—The chemotaxis of two pseudomonads, *Pseudomonas putida* AZ (Naph$^+$) and *P. putida* AZ (Naph$^-$), differing in their ability to metabolize naphthalene was studied by the known capillary method of Adler and the densitometric method devised in our laboratory. The migration of *P. putida* AZ (Naph$^+$) cells toward increasing levels of naphthalene was accompanied by the formation of a migrating front of converted naphthalene. *P. putida* AZ (Naph$^-$) cells also exhibited positive chemotaxis to naphthalene, but they did not form the front of converted naphthalene. The analysis of experimental data in terms of a kinetic model of bacterial chemotaxis showed that the densitometric method is a potential tool for studying bacterial chemotaxis to hydrophobic organic substances.

Key words: bacterial chemotaxis, technogenic pollution, contaminants, bioremediation, densitometry, naphthalene.

Bacterial chemotaxis is the migration of motile cells toward elevated levels of attractant chemicals or away from elevated levels of repellents. The mechanism of this phenomenon is well understood [1, 2]. Chemotaxis plays an important part in bioremediation of the environment, since many pollutants, including cyclic and polycyclic organic compounds insoluble in water, are bacterial attractants and may serve as sources of carbon and energy for bacteria [3, 4].

The pollution of the environment with polycyclic aromatic hydrocarbons (PAHs) threatens nature and human health. One of the promising methods for bioremediation of soils polluted by PAHs is the use of microorganisms capable of degrading these pollutants. Although there is abundant literature concerning this problem, the effect of bacterial chemotaxis on the bioremediation of PAH-polluted soils is far from being clearly understood.

The aim of this work was to study the chemotaxis of naphthalene-degrading bacteria to this PAH by the capillary method of Adler [5] and by the method of vertical densitometry devised in our laboratory [6].

MATERIALS AND METHODS

Experiments were carried out with two strains of pseudomonads, *Pseudomonas putida* AZ (Naph$^+$) and *P. putida* AZ (Naph$^-$), the former being capable of degrading naphthalene. The strains were grown to the exponential growth phase, when the motility and chemotaxis of cells are maximum. Washed cells were suspended in the so-called CHE buffer (10 mM potassium phosphate, pH 7.0) [5]. The chemotaxis of the strains to naphthalene was studied by two methods, the capillary method of Adler and the densitometric method devised in our laboratory.

The capillary method of Adler. This method is widely used in laboratories to study bacterial chemotaxis [5]. A capillary tube with an inner diameter of about 0.1 mm is sealed at one end and filled with a chemoeffector (typically an attractant) dissolved in an appropriate buffer. The tube is dipped into a bacterial suspension containing about 10^6 cells/ml. After incubation for some time (usually 1 h), the chemotaxis of bacterial cells is evaluated from a comparison of the numbers of cells penetrating into this tube and the control tube, which is filled with buffer containing no attractant. The number of bacterial cells in capillary tubes is accurately determined by plating the contents of the tubes onto agar media.

The densitometric method. The densitometric method devised in our laboratory [6–8] deals with considerably higher cell densities (10^8–10^9cells/ml) and greater measuring volumes. The method consists in the densitometric analysis of a bacterial suspension (i.e., the determination of the spatial distribution of bacterial cells) and the observation of the formation and migration of bacterial bands in a special optical cuvette. Then, the relationship between the macroparameters of the bacterial population and the microparameters of individual cells in the population can be determined from the kinetic analysis of densitometric data. With

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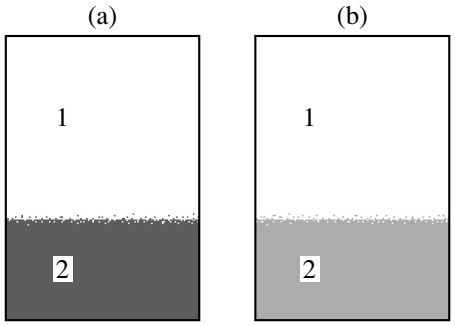

Fig. 1. Photographs showing the initial position of (1) naphthalene solution and (2) suspensions of (a) *P. putida* AZ (Naph$^-$) and (b) *P. putida* AZ (Naph$^+$) cells in optical cuvettes.

this method, 200 μl of a bacterial suspension with OD = 0.3 (this culture turbidity corresponds to a cell density of about 6×10^8 cells/ml) is carefully poured onto the bottom of a cuvette below the layer of a chemoeffector solution in CHE buffer. After 5–10 min (depending on the type of bacterial cells and the effector), the bacterial suspension begins to stratify, forming visually distinct bands of cells. The bands, whose formation obeys the same laws as in the case of the capillary method of Adler, either gradually disappear or slowly migrate at a velocity of about 1 cm/h. The dynamics of the concentration bands of bacteria can be visually observed and measured densitometrically.

The kinetic model of bacterial chemotaxis. The data obtained were analyzed in terms of a diffusion approximation of the kinetic model of bacterial chemotaxis [9, 10]:

$$\underbrace{\frac{\partial b}{\partial t}}_{(a)} - \underbrace{\frac{5v^2}{3\gamma_0}\frac{\partial^2 b}{\partial x^2}}_{(b)} + \underbrace{\frac{v}{3}\left(\Psi\frac{\partial b}{\partial x} + \frac{\partial \Psi}{\partial x}b\right)}_{(c)} = 0, \quad (1)$$

where b is the cell concentration along an optical cuvette; t is the time; v is the velocity of swimming bacterial cells; γ_0 is the frequency of change in the direction of cell motion in a chemically homogeneous medium; and Ψ is a chemotaxis function given by the formula:

$$\Psi = \frac{\delta K_D}{(K_D + C)^2}\frac{\partial C}{\partial x}v, \quad (2)$$

where δ is an empirical coefficient; C is the concentration of a chemical effector; and K_D is a constant accounting for the binding of the chemoeffector to its receptor.

If the effector can be metabolized by bacterial cells, equation (1) should be supplemented by the following equation that describes the diffusion and consumption of the effector:

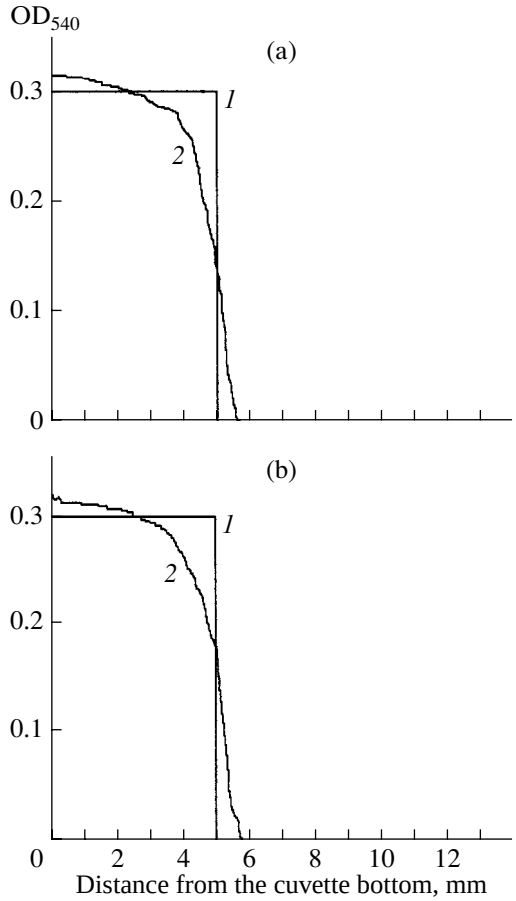

Fig. 2. Densitograms showing (*1*) the theoretical and (*2*) actual distribution of (a) *P. putida* AZ (Naph$^-$) and (b) *P. putida* AZ (Naph$^+$) cells in optical cuvettes at zero time (t = 0 min).

$$\frac{\partial C(x,t)}{\partial t} = -K(C)b(x,t) + D\frac{\partial^2 C(x,t)}{\partial x^2}, \quad (3)$$

where D is the diffusion coefficient of the effector and $K(C)$ is the rate at which the effector is consumed by bacterial cells.

Equation (1) describes the locomotive behavior of bacterial cells in a linear chemical gradient. The term (a) of this equation defines the temporal dynamics of the concentration of bacterial cells, the term (b) describes the proper chaotic wandering of motile bacterial cells in a chemically homogeneous medium, and the term (c) gives the migration of bacterial cells toward elevated levels of an attractant. The last term depends on the affinity of cell receptors to the attractant molecules and on the steepness of their gradient. Thus, the resultant motion of cells in a bacterial population is the sum of the random wandering of bacterial cells and their directional migration toward elevated concentrations of the attractant.

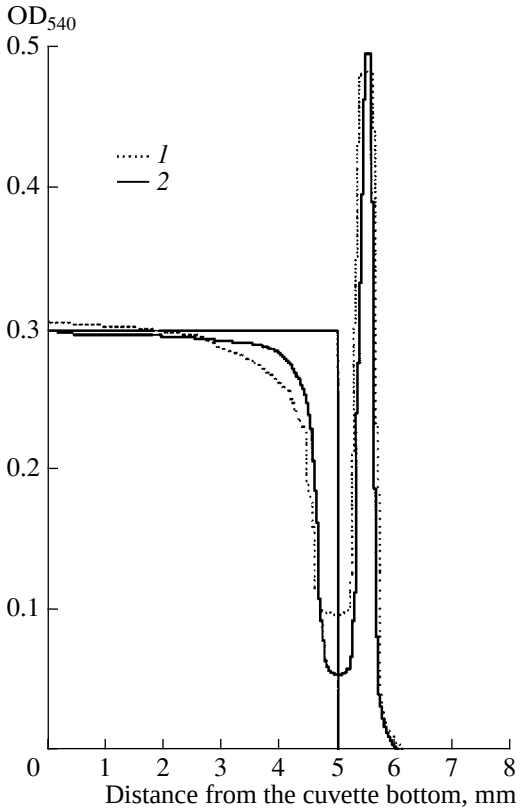

Fig. 3. Densitograms showing (*2*) the theoretical and (*1*) actual distribution of *P. putida* AZ (Naph$^+$) cells in an optical cuvette at the time instant $t = 0$ min. The distribution of *P. putida* AZ (Naph$^-$) cells was almost the same.

RESULTS AND DISCUSSION

The photographs in Figure 1 show the distribution of (a) *P. putida* AZ (Naph$^-$) and (b) *P. putida* AZ (Naph$^+$) cells in an optical cuvette at the instant the bacterial suspension is poured onto the bottom of the cuvette below the layer of a naphthalene solution. The corresponding densitograms of the cuvette contents are presented in Figs. 2a and 2b (the straight lines *1* in these figures show the theoretical distribution of bacterial cells). At zero time ($t = 0$), the distribution of cells of both strains was the same (curves *2* in Figs. 2a, 2b). The diffusion of the interface between the bacterial suspension and the naphthalene solution is due to the proper motility of cells and convective flows at the interface. Some accumulation of bacterial cells near the cuvette bottom can be explained by the action of gravity. If the buffer contained no naphthalene, such a distribution of bacterial cells along the cuvette axis, with the width of the interface not exceeding 1 mm, remained virtually unchanged for 1–2 h (data not presented).

If, however, the buffer contained naphthalene, immediately after 10 min of incubation, the populations of cells of both strains began to stratify (Fig. 3). Within a sufficiently short period of time ($t < 15$ min), the distribution patterns of *P. putida* AZ (Naph$^+$) and *P. putida* AZ (Naph$^-$) cells were almost the same, which can be explained by the fact that the consumption of naphthalene by the strain AZ (Naph$^+$) within this period of time is too small to affect the naphthalene gradient.

As is evident from equation (2), the chemotaxis function Ψ has maximum values at relatively low concentrations of the effector (close to the binding constant of the cell receptor, K_D) and at its steep gradient (i.e., at high values of $\partial C/\partial x$). These conditions are satisfied at the interface between the bacterial suspension and the naphthalene solution within the initial period of time.

The further behavior of cells depends on several factors. First of all, as pseudomonads are aerobes and oxygen serves as an attractant for them, they can experience the so-called aerotaxis effect. The presence of dissolved molecular oxygen in the naphthalene solution brings about the formation and development of an additional band of migrating bacterial cells. For illustration, Fig. 4 shows the photographs of optical cuvettes with (a) *P. putida* AZ (Naph$^-$) and (b) *P. putida* AZ (Naph$^+$) cells, which were taken 1 h after the bacterial suspension was poured onto the cuvette bottom below the naphthalene solution that was not degassed. The densi-

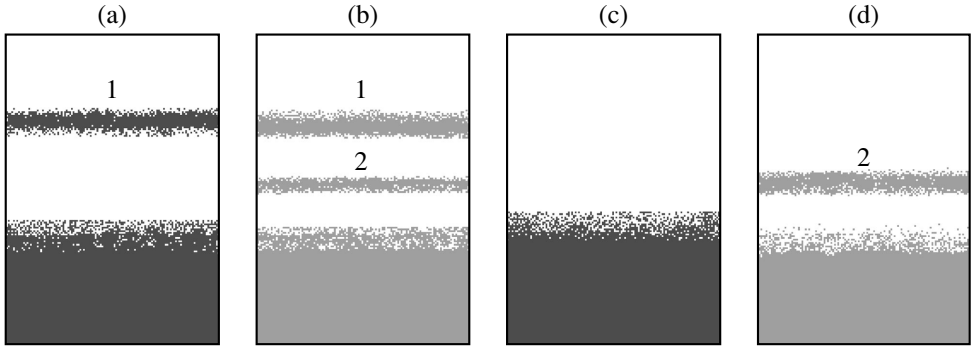

Fig. 4. Photographs showing the distribution of (a, c) *P. putida* AZ (Naph$^-$) and (b, d) *P. putida* AZ (Naph$^+$) cells in optical cuvettes at the time instant $t = 60$ min in (a, b) the presence and (c, d) absence of dissolved oxygen. In the absence of oxygen, both strains did not form the migrating band 1. Unlike the *P. putida* AZ (Naph$^+$) cells, the *P. putida* AZ (Naph$^-$) cells did not form the migrating band 2 either (see text for explanation).

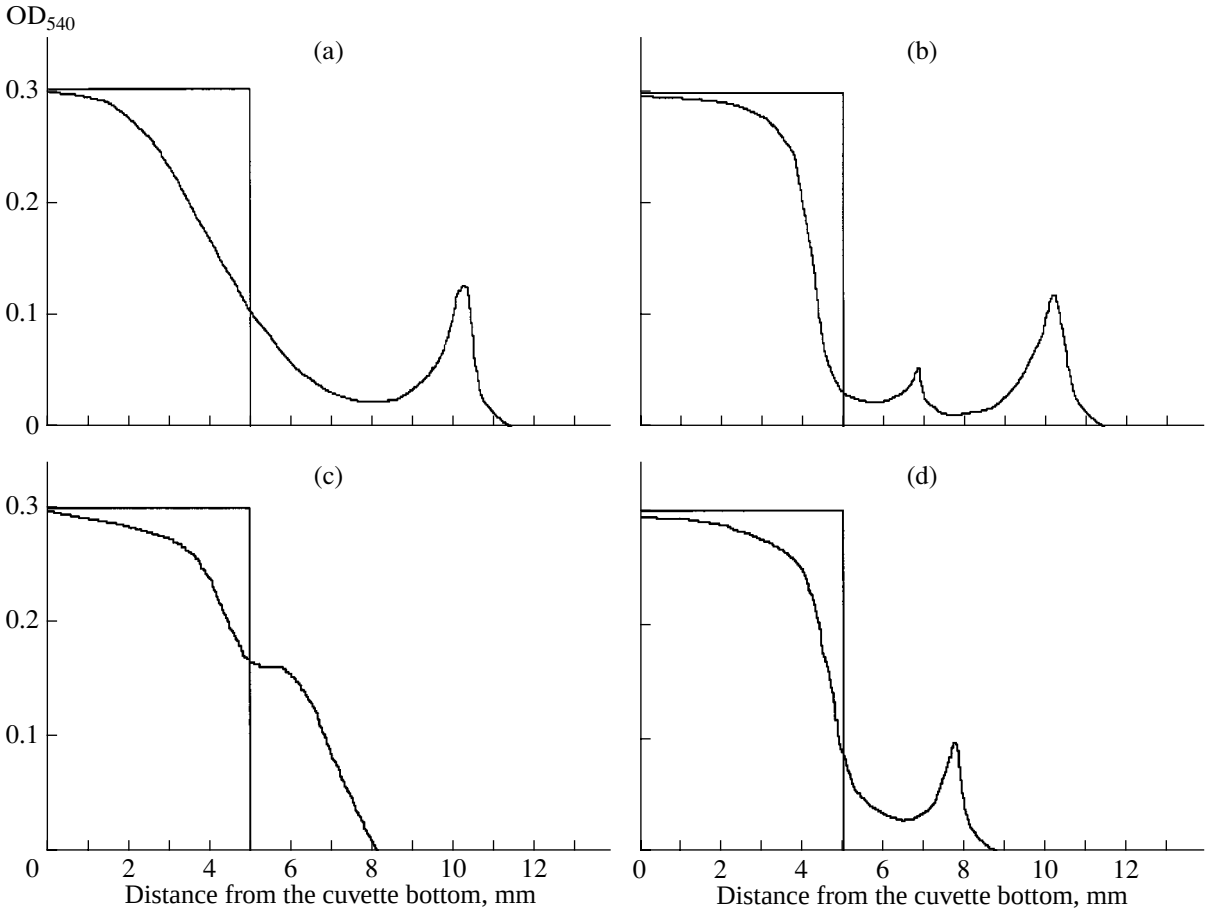

Fig. 5. The densitograms of the distribution of (a, c) *P. putida* AZ (Naph$^-$) and (b, d) *P. putida* AZ (Naph$^+$) cells in optical cuvettes at the time instant t = 60 min in (a, b) the presence and (c, d) absence of dissolved oxygen.

tograms corresponding to these photographs are shown in Figs. 5a and 5b. In the case of Naph$^-$ cells, there is one migrating band (1), whereas Naph$^+$ cells produce two bands (1 and 2), which migrate at different velocities. If the naphthalene solution was degassed and experiments were carried out using a hermetically sealed optical cuvette, the faster migrating cell bands 1 were not observed (Figs. 4c, 4d, 5c, 5d). Consequently, the formation of bands 1 was caused by aerotaxis (i.e., the chemotaxis of bacterial cells to oxygen dissolved in naphthalene solution).

Let us now consider the reason for the absence of the chemotactic band of Naph$^-$ cells. The population of these cells migrated toward elevated levels of naphthalene without forming a distinct band of migrating cells. Such chemotactic behavior of non-naphthalene-utilizing cells is in agreement with theory, which predicts that a distinct band of cells migrating to an attractant is formed only when this attractant is a utilizable source of energy (in other words, a metabolizable substrate) for these cells [8]. In such a case, the velocity of the cell band migration is determined by the substrate consumption rate. Bacterial cells behave as if they seek the front of the metabolizable attractant.

The agreement of theory with experiment improves the higher the accuracy of measurement of the microscopic parameters of cells migrating toward an attractant.

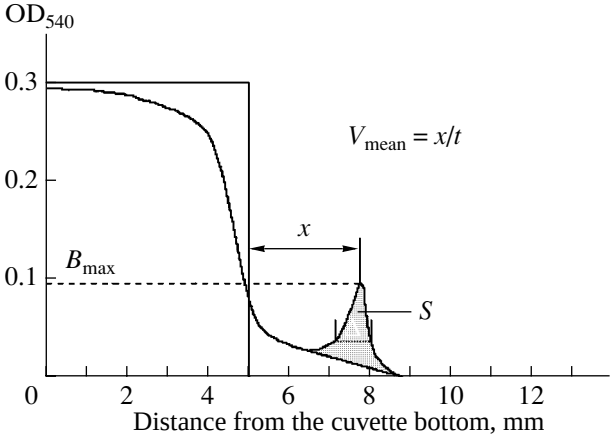

Fig. 6. Diagram illustrating the measurement of chemotactic parameters in the densitometric method for studying chemotaxis.

Densitometric parameters characterizing the chemotaxis of *P. putida* AZ ($Naph^+$) and *P. putida* AZ ($Naph^-$) cells to naphthalene

Parameter	Incubation time *t* (min)						
	0	10	20	30	40	50	60
$Naph^-$ (migrating band is not formed)							
Front displacement *X* (mm)	0	1.5	2.0	2.5	2.75	3.0	3.2
Front velocity $V_{mean} = X/t$ (mm/h)	9	6	5	4	3.5	3.2	3
Front velocity $v = dX/dt$ (mm/h)	9	3	3	1.25	1.25	1.2	1.0
$W \times 10^{-7}$ cells	0.3	1.0	1.5	2.0	2.3	2.4	2.5
$Naph^+$ (migrating band is formed)							
Band displacement *X* (mm)	0	0.5	1.0	1.5	2.0	2.5	3.0
Band velocity $V_{mean} = X/t$ (mm/h)	3	3	3	3	3	3	3
Band velocity $v = dX/dt$ (mm/h)	3	3	3	3	3	3	3
B_{max} (OD_{540})	–	0.5	0.3	0.25	0.2	0.15	0.1
Δ (mm)		0.5	0.8	0.9	1.0	1.0	1.0
$S \times 10^{-7}$ cells	0.3	0.8	0.1.5	1.5	1.3	1.2	1.1
$W \times 10^{-7}$ cells	0.4	1.2	1.7	2.1	2.2	2.3	2.4

The major parameters of the densitometric method are as follows: the displacement of the cell band (or the bacterial population front when the band is not formed) relative to the initial interface between the bacterial population and the effector solution, *X*; the velocity of the migrating band of cells or the velocity of the bacterial front displacement, *V*; the maximum concentration of cells in the band, B_{max}; the number of bacterial cells in the band, *S*; the total number of cells migrating toward an attractant, *W*; and the band width, Δ. Figure 6 schematically depicts these parameters, whereas their dynamics for *P. putida* AZ ($Naph^+$) and *P. putida* AZ ($Naph^-$) cells chemotactically migrating toward naphthalene is presented in the table. The velocity of the migrating band of cells can be determined either from the displacement of the peak concentration of bacterial cells relative to the initial interface between the bacterial population and the effector solution (the so-called mean velocity $V_{mean} = X/t$) or as the local velocity of the completely formed cell band, $v = dX/dt$. Both velocities may change during a particular experiment.

The band width Δ is measured at a height $1/e$ relative to the baseline defined as shown in Fig. 6. The number of bacterial cells in the band is calculated as the area of the shaded peak (Fig. 6).

The data presented in the table show the difference in the chemotactic behavior of cells capable and incapable of metabolizing the attractant ($Naph^+$ and $Naph^-$ cells, respectively). It can be seen from the table that, initially, the velocity of the migrating front of $Naph^-$ cells considerably exceeds the velocity of the migrating band of $Naph^+$ cells. In the course of chemotaxis, however, the velocity of the migrating band of $Naph^+$ cells remains constant, whereas the velocity of the migrating front of $Naph^-$ cells gradually declines. The decline in the velocity of $Naph^-$ cells can be explained by the fact that the naphthalene gradient, in this case, changes little (due to the diffusion of naphthalene molecules), so that migrating bacterial cells leave the zone of the gradient and the force causing them to migrate disappears. The constant velocity of migrating $Naph^+$ cells is due to the fact that they consume naphthalene, so that the concentration gradient of naphthalene shifts along the cuvette axis together with the migrating cells, which concentrate in the zone of the naphthalene gradient. In the course of migration, the peak concentration of cells in the band gradually declines, the band width increases, and the total number of cells in the band diminishes.

A comparison of the data obtained by three methods (the capillary method of Adler, the densitometric method, and the method of chemotactic rings formed on the surface of semiliquid agar) shows that the third method is free of the interfering effect of aerotaxis, since, in this case, bacterial cells are in permanent contact with the air. The major results obtained by the three methods are similar. The advantages of the densitometric method are its relative simplicity, informativeness, and promptness. Furthermore, it allows the temporal dynamics of the chemotactic behavior of cells to be investigated and makes it possible to automatize and computerize the process of observation of cell chemotaxis.

Experiments also showed that the cultivation temperature of cells influenced the velocity of cell migration toward elevated levels of naphthalene (this velocity was 3.2–4-fold higher at 2°C than at 8°C), but influenced neither the threshold concentration of naphthalene necessary for the development of the chemotactic response of cells nor the qualitative characteristics of chemotaxis. It should be noted that the strains Az $Naph^+$ and $Naph^-$ exhibited chemotaxis not only to naphthalene but also to some other metabolizable compounds.

In this study, we did not touch the important problem of signalling pathways in the chemotactic response of pseudomonads to naphthalene, which may involve (1) the general system of the reversible methylation of transmembrane proteins responsible for the chemotaxis of cells to amino acids and sugars, (2) the enzyme II of the phosphotransferase system, (3) the protein complex of the flagellar motor, and (4) the protonmotive force generated by membranes [1]. Furthermore, the possibility cannot be excluded that the chemotactic response of cells to naphthalene is mediated by its intermediates or membrane lipoproteins.

To conclude, the densitometric method can be employed for investigating the chemotaxis of bacterial cells not only to naphthalene but also to other hydrophobic organic compounds, both metabolizable and nonmetabolizable by these cells.

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