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EXPERIMENTAL ARTICLES

Influence of Environmental Factors on the Chemotaxis of *Bradyrhizobium japonicum*

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Abstract—Dependence of motility and chemotaxis was studied in two strains of *Bradyrhizobium japonicum* upon several environmental factors. In both strains, chemotaxis was found to increase with an increasing concentration of the attractant (glucose) to 5.5×10^{-2} M. Both motility and chemotaxis reached their maximum in the two- to three-day cultures at neutral pH. The maximum motility of these bacteria occurred at 40°C. The maximum values of chemotaxis in these microorganisms were, however, observed at 20–25°C. Chemotaxis in acidic or alkaline media and at low temperatures was found to be markedly weaker. Nonoptimal values of these parameters in soil may be a limiting factor for the interaction of the given bacteria with soybean roots.

Key words: chemotaxis, *Bradyrhizobium japonicum*, effect of environmental factors on chemotaxis.

The symbiosis of rhizobia and leguminous plants is known to have a significant influence on the development and growth of legumes [1, 2]. The first step in the formation of such a symbiosis is the release of exudates by leguminous plants, which causes the movement of rhizobia to their roots [3, 4]. This movement appears to be a factor controlling the interaction of root-nodule bacteria with the host plant. The essentials of chemotaxis are covered in a number of surveys [5–7].

Bacterial chemotaxis is known to be affected by a wide range of factors, including the bacterial population density, pH, the ambient temperature, and the presence of several chemical compounds [3, 4, 8–10].

Most of the previous works concerned with the chemotaxis of rhizobia were carried out on fast-growing root-nodule bacteria [3, 7, 11, 12]. Meanwhile, the available literature lacks consistent evidence about the effect of physical factors on the chemotaxis of *Bradyrhizobium japonicum*, and there are only random reports on the action of several chemical compounds (aromatic compounds and flavonoids) [13, 14]. The latter data, however, fail to produce a comprehensive picture of chemotactic properties of the given microorganism. The goal of this work was, therefore, to investigate the effect of several environmental factors on the chemotaxis of *B. japonicum*.

MATERIALS AND METHODS

The two bacterial strains used were *B. japonicum* 634b and 10K, which were provided by the museum of live cultures of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. Microorganisms were cultured in 750-ml Erlenmeyer flasks, each containing 100 ml of liquid nutrient medium composed of (g/l) $K_2HPO_4 \cdot 3H_2O$, 0.25; KH_2PO_4, 0.25; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.1; $((NH_4)_2SO_4$, 0.5; mannitol, 10.0; yeast autolysate, 1.0; and distilled water. The pH of the medium was 6.5–6.7. The medium was inoculated with bacterial suspension (1 vol %) and the flasks were incubated at 28°C for 3 days.

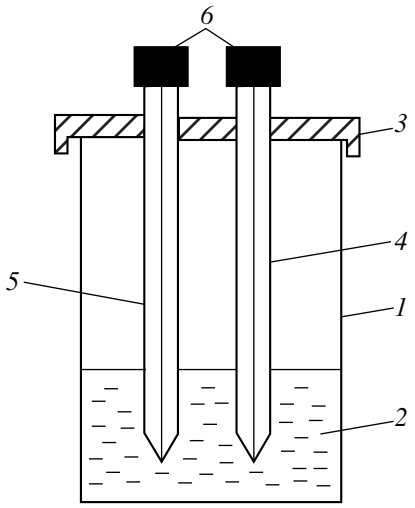

Fig. 1. Diagram of a chamber for studying bacterial chemotaxis: (*1*) vessel for bacterial suspension; (*2*) bacterial suspension; (*3*) tube holder plate; (*4*) capillary tube without the attractant; (*5*) capillary tube with the attractant; and (*6*) silicon tube with a rubber plug.

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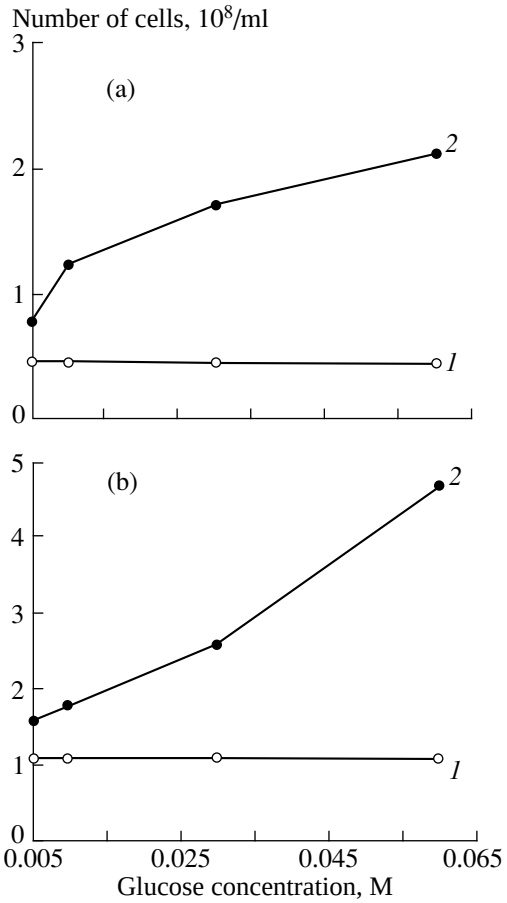

Fig. 2. Dependence of chemotactic properties of (a) *B. japonicum* 10K and (b) 634b on glucose concentration in the medium. The number of bacteria in microcapillaries (*1*) without the attractant and (*2*) with different concentrations of glucose in the medium. The original bacterial suspension contained 3×10^8 cells/ml.

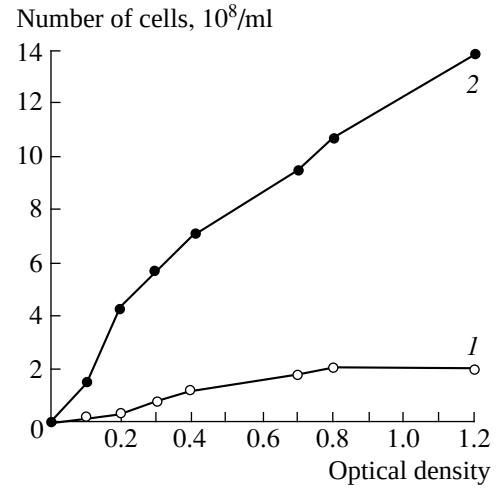

Fig. 3. The number of bacteria in microcapillary tubes (*1*) without and (*2*) with the attractant as a function of the population density of *B. japonicum* 634b in the suspension.

The bacteria were washed three times to remove the culture medium by centrifugation (2000*g*, 15 min), and were then resuspended in potassium phosphate buffer (0.01 M, pH 7.0) that was prepared using bidistilled water. In order to restore bacterial motility, the suspension was thermostatted at 28°C for 60 min.

The chemotactic properties of bacteria were studied in capillary glass tubes with an internal diameter of 0.84 mm. One end of each capillary was left open and the other sealed with a silicon tube and a rubber plug (Fig. 1). Using a syringe and by puncturing the plug with an injection needle, the capillaries were filled with buffer solutions, which, depending on the test, contained or did not contain glucose (5.6×10^{-2} M), which was introduced as the effector.

Filled capillary tubes were fixed in a vertical position in beakers using plates with holes made to fit the tubes (Fig. 1). Each beaker contained 110 ml of the suspension of the microorganisms studied.

The beakers with filled capillaries were incubated at 28°C for 60 min [8]. Then, a capillary tube was taken out of the beaker, its edge was rinsed with sterile water and blotted with filter paper, and 0.01 ml of the contents of the capillary bottom part was put on a slide. The suspension was evenly spread over an area of 4 cm^2. This smear was then dried and fixed in a flame, and the bacteria were counted directly in a Biolar polarizing interference microscope (Poland). Microorganisms were counted in 50 microscopic fields, each containing 15 to 30 bacteria. Each measurement was triplicated and each experiment was done at least three times.

The degree of bacterial motility was measured in terms of the number of cells found in the analyzed part of the tube containing no attractant. Bacterial chemotaxis was estimated from the difference in the number of cells in 0.01 ml of the buffer solution in capillaries containing and not containing the attractant.

In tests to study the effect of pH on chemotaxis, capillary tubes were filled with the buffer of the above composition either containing or not containing the effector and with pH varying between 6 and 8.

In tests to investigate the temperature dependence of the chemotaxis of the given bacteria, after the motility restoration step, the beakers with the suspension were kept at different temperatures (15–45°C) for 30 min to allow the bacteria to attain the required temperature. Then, filled capillary tubes were placed in the suspension and incubated for 1 h at the above temperatures.

The results of the experiments were statistically processed [15]. The half-width of the 95% confidence interval for the mean of all test replications amounted to 6.5% of the mean.

RESULTS AND DISCUSSION

Given that glucose is one of the most readily available sources of carbon and energy for *B. japonicum* [16], it was used as an attractant in our study of chemotaxis in this microorganism. The population density of *B. japonicum* 10K in capillaries filled with the medium without the attractant was estimated to be 0.45×10^8 cells/ml. For *B. japonicum* 634b, the number of bacteria under similar conditions was 1.1×10^8 cells/ml (Fig. 2). The population density of both strains in the capillary tubes increased with the concentration of glucose in the chemotaxis medium and attained maximum values of 2.13×10^8 for strain 10K and 5.4×10^8 for strain 634b at a glucose concentration of 5.6×10^{-2} M (Fig. 2). Therefore, in our subsequent experiments, the latter concentration of glucose was used.

The numbers of cells in the microcapillary tubes both with and without the attractant were shown to be mostly determined by the population density in the original suspension. The higher this density, the higher the content of microorganisms in capillary tubes both in the presence and in the absence of the attractant (Fig. 3). A similar dependence upon the population density was previously reported for *Methylosinus trichosporium* OB3b [9].

It should be noted that the strength of chemotaxis is sometimes expressed in terms of the chemotactic index [9], which is defined as the ratio of the number of bacteria in a capillary containing an effector to their number in a capillary with no effector. The value of this index is greater than unity if the effector is an attractant and smaller than unity if it is a repellent. It can be inferred from Fig. 3 that the chemotactic index for a diluted suspension was as large as 10, decreasing to 5–7 at increased population densities.

The motility and chemotactic capacity of *B. japonicum* were found to depend on the culture age. In *B. japonicum* 634b, these values reached their maximum in the two-day cultures. Meanwhile, in *B. japonicum* 10K, the motility and chemotaxis peaked in the three-day cultures (Fig. 4). These quantities showed a decline during further cultivation, and this could be due to a possible change in the physiological state of bacteria occurring in the cultivation process.

The obtained results suggest that the population density of bacteria and their age should be standardized in chemotaxis assays. Accordingly, suspensions of three-day old cultures of *B. japonicum* with roughly equal population densities (0.28–0.30 optical density units) were used in our tests to obtain consistent chemotactic responses of the bacteria and to be able to directly count the bacteria.

The dependence of motility and chemotaxis of strains 634b and 10K of *B. japonicum* on the medium pH is shown in Fig. 5. Both variables attained their maximum values at pH 6.5–7.5. As one can see, chemotaxis of these strains decreased in acid (pH 6.0) and alkaline media (pH 8.0), most notably at pH 8.0.

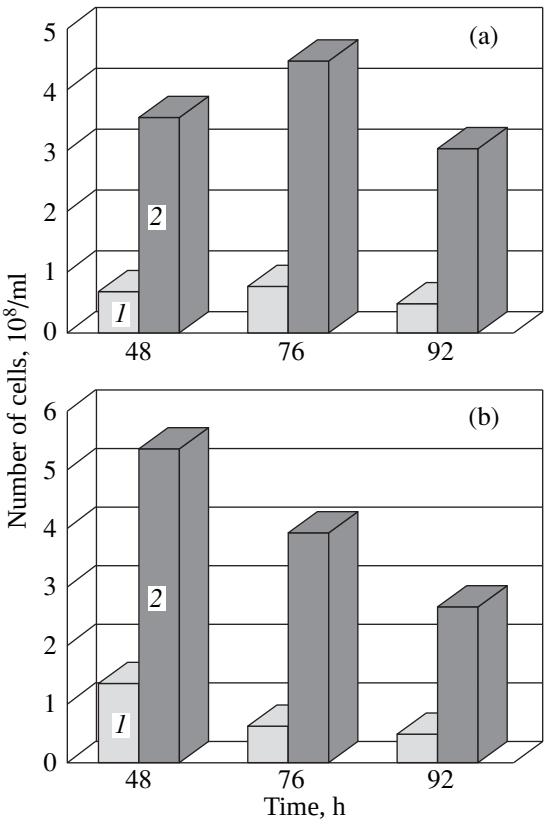

Fig. 4. Effect of the culture age on the motility and chemotactic properties of (a) *B. japonicum* 10K and (b) 634b: the number of bacteria in capillaries (*1*) without and (*2*) with the attractant.

This is an indication that, in acid and alkaline soils, the chemotaxis of *B. japonicum* to soybean seeds can be a limiting factor for the development of symbiosis between soybean plants and rhizobia.

The effect of temperature on the motility and chemotaxis of *B. japonicum* is shown in Fig. 6. Motility of strain 634b was found to gradually increase with temperature to its maximum value at 40°C. Motility of strain 10K exhibited a less notable dependence on the incubation temperature. With glucose introduced into the medium, the chemotactic response of this strain grew markedly when the temperature was changed from 15 to 20°C and attained a maximum at 25°C. Chemotaxis to glucose in *B. japonicum* 634b showed a peak at 20–25°C, and the range of optimum temperatures for this strain was wider (20–35°C) than for strain 10K. At higher incubation temperatures, chemotaxis shown by these bacteria gradually decreased, but even at 40°C, it remained stronger than at 15°C.

Based on our findings of how chemotaxis in *B. japonicum* depends on the ambient temperature, it is possible to estimate the effect of temperature on the interaction between this bacterium and plants in agricultural production. Thus, for the northern and middle parts of Ukraine, it was suggested that soybean be sown

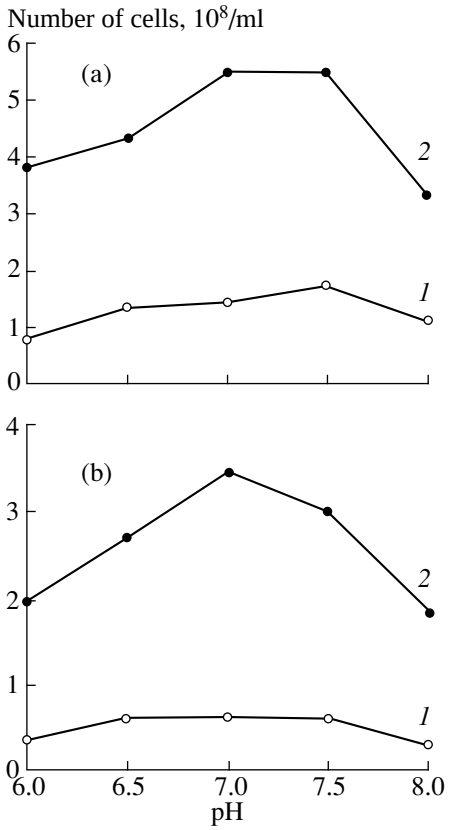

Fig. 5. Effect of the medium pH on the motility and chemotactic properties of *B. japonicum* 10K (a) and 634b (b): the number of bacteria in capillaries (*1*) without and (*2*) with the attractant.

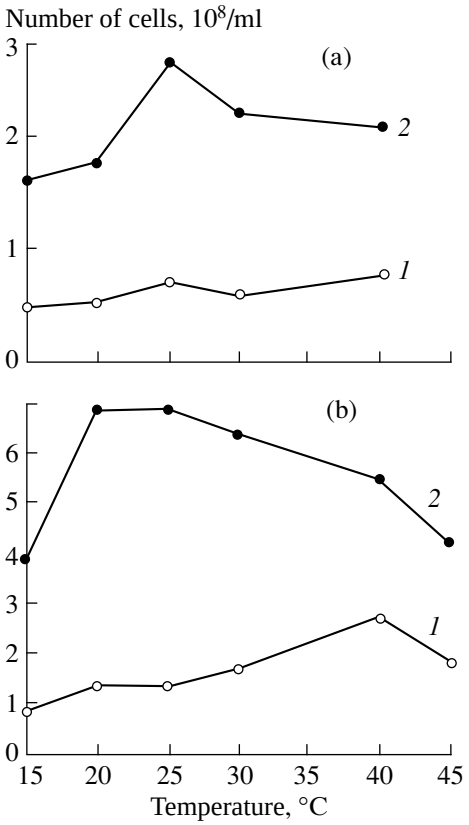

Fig. 6. Motility and chemotactic properties of *B. japonicum* 10K (a) and 634b (b) as dependent on the incubation temperature: the number of bacteria in capillaries (*1*) without and (*2*) with the attractant.

in the second part of April through the first part of May, when the soil temperature increases to 10°C [17]. A recently proposed method of bacterial soybean treatment consists of introducing the bacterial preparations into furrows and row-spacings during the process of sowing [18, 19]. In the southern regions of Ukraine and Russia, where the soil becomes sufficiently warm by this time of the year, the chemotaxis of bacteria to soybean seeds under the given method of inoculum introduction may not be a factor limiting the interaction of *B. japonicum* with the plant.

On the other hand, our results show that chemotaxis is insignificant at low environmental temperatures. It seems likely, therefore, that for the early sowing of soy, the establishment of symbiosis between the plant and root nodule bacteria would be better promoted by directly applying the bacteria to the seeds before they are sown. Introducing bacterial preparations into furrows in not so warm soil (concurrently with sowing) in the form of granules or a suspension at some distance from the seed, will most likely fail to promote the interaction of bacteria with soybean plants, and thus may not have a positive effect on the crop.

In summary, optimal values of several environmental parameters for chemotaxis in *B. japonicum* were determined in this study. The obtained results can be used to predict the activities of these bacteria in soil under the given conditions of their application.

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