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EXPERIMENTAL ARTICLES

Chemotaxis and Growth of *Bradyrhizobium japonicum* in the Presence of Fine-Dispersed Silica

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Abstract—The chemotactic properties of the soybean nodule bacterium *Bradyrhizobium japonicum* were studied in the presence of synthetic fine-dispersed materials. It was shown that fine-dispersed silica (FDS) and its variety modified with aluminum oxide (MFDS) reduce bacterial chemotaxis to glucose. In addition, FDS increases the irregular motility of *B. japonicum*, and MFDS decreases it. This is in agreement with the effect of the materials on the rate of nodule bacterium growth.

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Key words: chemotaxis, Bradyrhizobium japonicum, fine-dispersed silica.

We showed previously that natural fine-dispersed materials (argillaceous minerals montmorillonite and palygorskite) induced irregular motility and reduced chemotaxis in soybean nodule bacteria [1]. Argillaceous minerals are alumosilicates that contain 70% silica and aluminum oxide [2]. This prompted us to study the effect of individual components of these composite minerals on bacteria. In our opinion, such study can be best performed with synthetic fine-dispersed materials based on silica. Such materials are chemically pure and resistant to biological and thermal degradation [3]. Fine-dispersed silica varieties are broadly used in science and industry. In particular, they are recommended for manufacturing preparations of nitrogen-fixing organisms in order to increase their biomass yield [4, 5].

Therefore, the goal of our work was to study the chemotaxis and growth of *Bradyrhizobium japonicum* in the presence of fine-dispersed silica, as well as a variety of it modified with aluminum oxide.

MATERIALS AND METHODS

Experiments were performed with slow-growing strains of soybean nodule bacteria, *Bradyrhizobium japonicum* 634b (industrial strain) and 604K (highly virulent inactive strain). The strains were kindly provided by Dr. N.Z. Tolkachev (Crimean Branch of the Institute of Agricultural Microbiology, Ukraine). The microorganisms were cultivated on mannitol–yeast extract medium [6] at 28°C until the middle of the logarithmic growth stage.

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The fine-dispersed silica (FDS) and fine-dispersed silica modified with aluminum oxide (MFDS) used in the study were provided by the Institute of Surface Chemistry, Ukraine. The particle size of the materials varied within 5 to 40 nm [7].

Contact interactions between particles of the dispersed materials and bacterial cells were studied by microelectrophoresis. The method is based on the fact that adsorption of fine solid-phase particles on the surface of bacterial cells alters the electrophoretic mobility (EM) in such a way that the EM value of such bacterium–solid complexes approaches (increases or decreases) that of solid particles [8].

The chemotactic properties of bacteria were studied in the presence of FDS and MFDS. The fine-dispersed materials were added to the bacterial suspension in the colloidal form (at concentrations from 0.05 to 1.00 g/l), mixed, and incubated for 30 min for establishment of contact. After that, chemotaxis was measured by a capillary method [6]. The bacterial suspension contained 4.5×10^8 cells/ml. The motility of bacteria was estimated from the number of cells entering capillary tubes with potassium–phosphate buffer (0.01 M, pH 7.0). In this case, motility is viewed as overall irregular motion, Brownian motion, and so on. Estimates of chemotactic response were derived from the difference between the numbers of cells in capillary tubes filled with glucose-supplemented buffer (5.6×10^{-2} M glucose) and the same buffer without glucose.

The effect of FDS and MFDS on the growth rate of *B. japonicum* 634b was estimated from the increase in the cell population after cultivation in liquid mannitol–yeast extract medium. The solid materials were added to flasks with 100 ml of this medium to concentrations

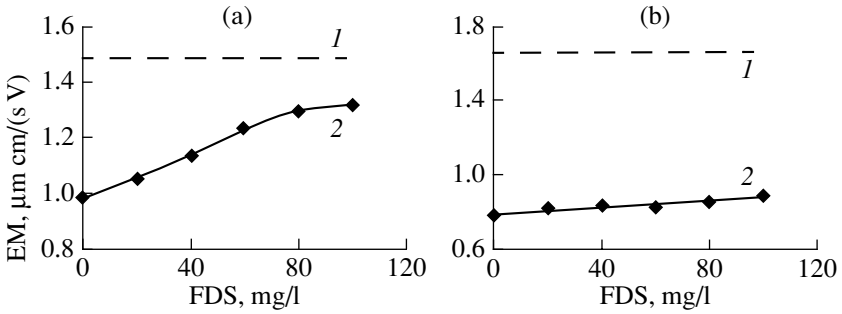

Fig. 1. Dependence of the electrophoretic mobility (EM) of *B. japonicum* 634b on the concentrations of (a) fine-dispersed silica (FDS) and (b) fine-dispersed silica modified with aluminum oxide (MFDS): *1*, EM of FDS or MFDS particles; *2*, EM of *B. japonicum* 634b cells.

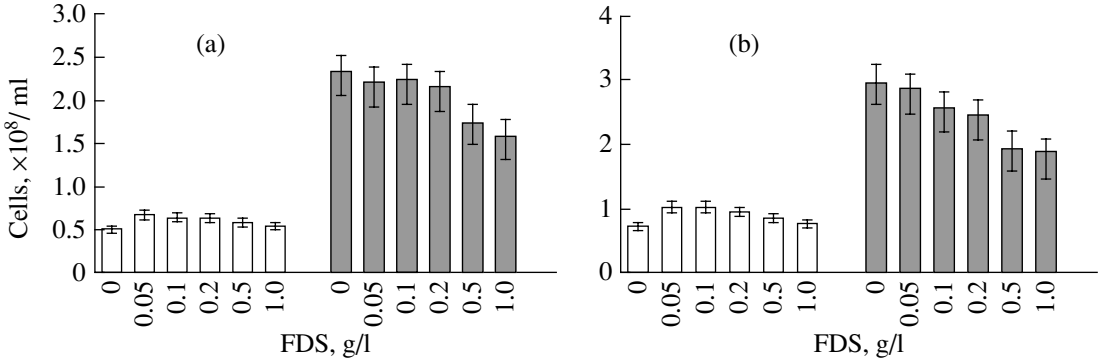

Fig. 2. Effect of fine-dispersed silica (FDS) on the chemotaxis of (a) *B. japonicum* 634b and (b) *B. japonicum* 604K estimated as the numbers of cells in capillary tubes with (□) potassium–phosphate buffer and (■) buffer + 5.6×10^{-2} M glucose.

of 0.05, 0.1, 0.2, 0.5, and 1.0 g/l. Control flasks were incubated without solids. When the bacteria reached the middle of the log phase (72 h of cultivation), the titer of colony-forming units (CFUs) was determined by tenfold serial dilutions by inoculation on solidified mannitol–yeast extract medium.

Statistical evaluation of the results was performed according to [9].

RESULTS AND DISCUSSION

Interactions of microorganisms of various taxa with fine-dispersed materials are accompanied by adsorption of solid particles on the cell surface [10, 11]. Microelectrophoretic experiments confirmed the adsorption of FDS particles on the surface of *B. japonicum* 634b cells (Fig. 1a) as the electrophoretic mobility of the bacteria changed, approaching that of FDS particles. The adsorption of MFDS particles was less pronounced, because in their presence, the EM of the bacteria did not change significantly (Fig. 1b).

At all silica concentrations studied, its interaction with *B. japonicum* 634b was accompanied by stimulation of microorganism motility (Fig. 2a). The greatest effect was observed at 0.05 g/l FDS. This concentration increased bacterial motility by 32%. At higher concentrations, the effect of FDS on the motility of bradyrhizobia decreased, with complete cessation taking place at 0.1 g/l. The interaction was also accompanied by a decrease in bacterial chemotaxis towards glucose, which was used as an attractant in our experiments. At 0.05–0.20 g/l FDS, the chemotactic response decreased insignificantly. An increase in silica concentration to 0.5 and 1.0 g/l caused a greater reduction of bacterial chemotaxis, by 26 and 33%, respectively (Fig. 2a).

Similar results were obtained in experiments where we assessed the effect of FDS on the motility and chemotaxis of the cells of *B. japonicum* strain 604K, which lack the ability to fix nitrogen (Fig. 2b). At 0.05 and 0.10 g/l FDS in the bacterial suspension, the flow of cells into capillary tubes with potassium–phosphate buffer was higher by 41% than in the microbial suspension without the fine-dispersed matter. The stimulating effect on the motility of *B. japonicum* 604 cells was an inverse increase in concentration (Fig. 2b).

The chemotactic response of *B. japonicum* 604K to glucose weakened on increasing FDS concentration in the bacterial suspension. As with strain 634b, the highest inhibiting effect on the chemotaxis of this microbial strain was observed at FDS concentrations of 0.5 and 1.0 g/l (35–37%) (Fig. 2b).

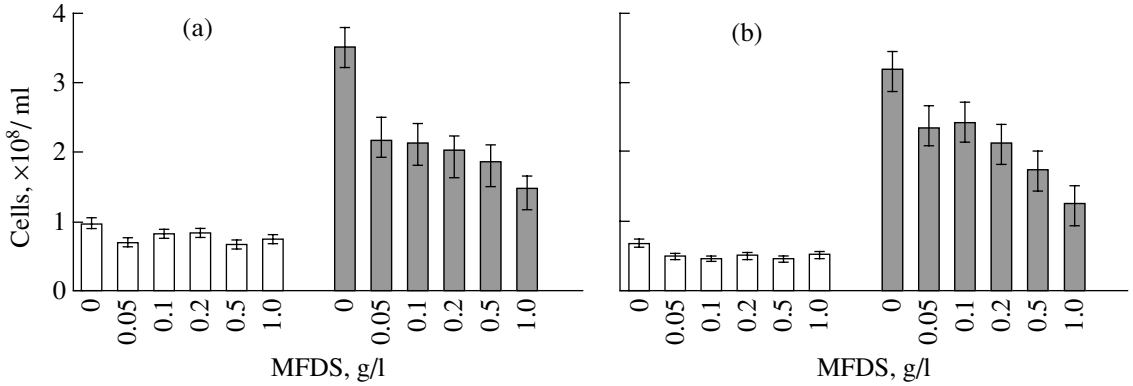

Fig. 3. Effect of fine-dispersed silica modified with aluminum oxide (MFDS) on the chemotaxis of (a) *B. japonicum* 634b and (b) *B. japonicum* 604K assessed by the number of cells in capillary tubes with (□) potassium–phosphate buffer and (■) buffer + 5.6×10^{-2} M glucose.

MFDS showed a negative effect on the motility of soybean nodule bacteria. At 0.05–1.00 g/l MFDS, the flow of *B. japonicum* 634b cells into capillary tubes with potassium–phosphate buffer was 14–28% less than that recorded for control tubes (Fig. 3a). Chemotaxis was also adversely affected by MFDS. Even minute MFDS concentrations (0.05 g/l) reduced chemotaxis by 38%. The inhibiting effect increased with MFDS concentration, reaching its maximum at 1.0 g/l. The content of cells in capillary tubes with glucose was 59% less than without MFDS (Fig. 3a).

It was found that the motility of *B. japonicum* 604K was also inhibited by MFDS. The negative effect was observed at all concentrations studied (Fig. 3b). The chemotactic response of *B. japonicum* 604K to glucose, as with *B. japonicum* 634b, became less pronounced on increasing MFDS concentration (Fig. 3b).

According to the literature, fine-dispersed silica species show both positive and negative effects on the activity of microorganisms of various taxa [4, 5, 10, 12, 13]. In our experiments, the motility of nodule bacteria was stimulated by FDS and inhibited by MFDS. Thus, it was of interest to study the effect of these synthetic fine-dispersed materials on *B. japonicum* growth. As the chemotactic responses in the presence of the fine-dispersed materials were similar in strains 634b (nitrogen-fixing) and 604K (unable to fix nitrogen), these experiments were performed only with *B. japonicum* 634b.

Our experiments showed that the effect of FDS on the growth of soybean nodule bacteria were concentration-dependent. The maximum growth-stimulating effect was observed at the lowest FDS concentration in the nutrient medium, 0.05 g/l. The titer of CFUs increased by 29.8% (Table 1). The increase in FDS concentration produced less-pronounced growth-stimulating effects: CFU titers in media with 0.1 and 0.2 g/l FDS was almost the same as in controls (without the fine-dispersed matter). Further increase in FDS concentration to 0.5 and 1.0 g/l inhibited the growth of *B. japonicum* (Table 1).

MFDS adversely affected the growth of soybean nodule bacteria at all concentrations studied (Table 2). The drop in CFU titers correlated to increased FDS concentrations.

Thus, the lowest FDS concentration studied stimulated the growth of *B. japonicum* 634b. The same concentration of this material stimulated the growth of some other microbial species [10, 12]. It is exactly at

Table 1. Effect of fine-dispersed silica (FDS) on the growth of *B. japonicum* 634b

Content of FDS in the nutrient medium, g/l	Bacterial titer in the culture liquid	
	cells $\times 10^8$/ml	% of the control
0.00 (control)	5.77 ± 0.10	100
0.05	7.49 ± 0.80	129.8
0.10	5.73 ± 0.25	99.3
0.20	5.86 ± 0.32	101.6
0.50	0.71 ± 0.13	12.3
1.00	0.85 ± 0.08	14.7

Note: The titer of bacteria in the beginning of the experiment was 4.4×10^6 cells/ml.

Table 2. Effect of fine-dispersed silica modified with aluminum oxide (MFDS) on the growth of *B. japonicum* 634b

Content of MFDS in the nutrient medium, g/l	Bacterial titer in the culture liquid	
	cells $\times 10^8$/ml	% of the control
0.00 (control)	5.07 ± 0.52	100.0
0.05	4.49 ± 0.21	88.6
0.10	4.67 ± 0.38	92.1
0.20	4.50 ± 0.32	88.8
0.50	4.02 ± 0.46	79.3
1.00	3.99 ± 0.23	78.7

Note: The titer of bacteria in the beginning of the experiment was 2.95×10^6 cells/ml.

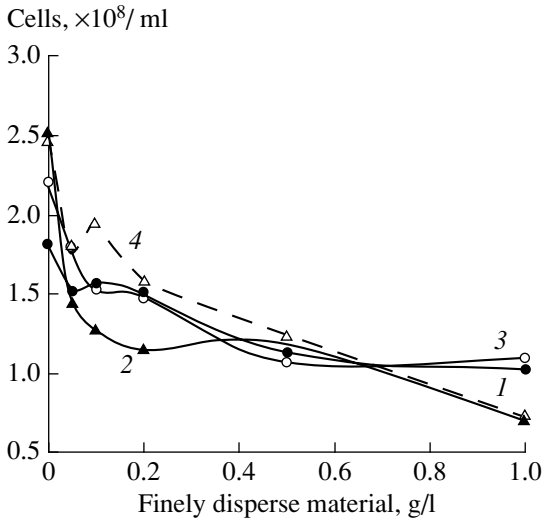

Fig. 4. Chemotaxis of (*1, 2*) *B. japonicum* 634b and (*3, 4*) 604K in the presence of (*1, 3*) fine-dispersed silica and (*2, 4*) fine-dispersed silica dioxide modified with aluminum oxide. The plot shows the difference between the numbers of cells in capillary tubes with glucose and with potassium–phosphate buffer.

this concentration that FDS exerted the maximum effect on the motility of our bacterial strain. In contrast, MFDS, which adversely affected the motility of nodule bacteria at all concentrations studied (0.05–1.00 g/l), inhibited their growth within the same concentration range. It is, therefore, conceivable that the effects of synthetic fine-dispersed silica on the motility of nodule bacteria and their growth are correlated.

Silica is widespread in the lithosphere, including soils, in the form of silicates and alumosilicates [3]. Therefore, they can affect the physiology of microorganisms in soils. Depending on the type and mode of introduction, these materials exert diverse effects on microorganism activity. The growth of *Agrobacterium radiobacter* 204 in a nutrient medium with pea pot liquor and sucrose was accelerated by addition of FDS but suppressed by MFDS [13]. Our experiments suggest that the surface of bacteria has specific silica-binding sites. When the interaction of MFDS with *B. japonicum* cells was insignificant (Fig. 1), it adversely affected their growth, motility, and chemotaxis. In contrast, the interaction of FDS with the cells was stronger: at low concentrations, their growth and motility were stimulated, whereas higher concentrations suppressed both types of activity. Thus, the effect of silica on the physiological properties of microorganisms is determined by both their concentration in the medium and the nature of their functional groups.

Moreover, fine-dispersed materials show a significant adsorption potential with regard to proteins and glycoproteins. Irreversible changes and degradation of membrane structures of blood cells under the effect of aluminum-containing silica were reported in [14]. Apparently, synthetic silica, as well as natural argillaceous materials, bind to and screen the chemotactic receptor proteins on the surface of *B. japonicum* (by either altering their conformation or degrading them). As a result, the chemotaxis to the attractant decreases (Fig. 4).

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