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EXPERIMENTAL ARTICLES

The Chemotactic Properties of *Bradyrhizobium japonicum* in the Presence of Natural Fine-Grained Minerals

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Abstract—The natural argillaceous minerals montmorillonite and palygorskite were found to enhance the motility of *Bradyrhizobium japonicum* cells and to slow down their chemotactic motion to glucose. The latter effect of the minerals is probably due to the adsorption of mineral particles on the cell surface and the blockade of the receptors that are responsible for the chemotactic behavior of the bacterium.

Key words: chemotactic properties, *Bradyrhizobium japonicum*, montmorillonite, palygorskite.

Bacteria that inhabit soil interact with fine-grained materials, including argillaceous minerals. Clay particles, which possess some specific surface properties (ion-exchange capacity, surface charge, and hydrophobic/hydrophilic balance [1]), may substantially influence the growth of soil bacteria [2].

The formation of a layer of fine particles on the surface of bacterial cells [3] may alter the surface properties of the cells [4, 5] and affect various cell functions, including cell chemotaxis.

The aim of this work was to study how natural fine minerals can affect the chemotaxis of the symbiotic nitrogen-fixing bacterium *Bradyrhizobium japonicum.*

MATERIALS AND METHODS

Experiments were performed with two strains (the industrial strain 634b and the highly virulent non-nitrogen-fixing strain 604K) of the slow-growing soybean-associated bacterium *Bradyrhizobium japonicum*. Both strain were generously provided by N.Z. Tolkachev from the Crimea Branch of the Institute of Agricultural Microbiology, Ukrainian Academy of Agrarian Sciences. The strains were grown in a mannitol–yeast extract medium [6] at 28°C to the mid-logarithmic phase.

The argillaceous minerals montmorillonite and palygorskite were obtained from the Cherkasskoe bentonite deposit. Montmorillonite is a laminated mineral composed of flocculent particles 0.05 μm in size. Palygorskite is a ribbonlike or fibrous aluminum silicate composed of acicular particles 0.2–0.5 μm in size [1].

To study the effect of the minerals on the chemotactic properties of the rhizobial strains, montmorillonite or palygorskite in the form of a colloidal suspension was added to a bacterial suspension at concentrations from 0.1 to 1.0 g/l. The suspensions were mixed and held for 30 min to ensure contact interactions between bacterial cells and mineral particles. Bacterial motion was studied by the capillary method [6]. Bacterial motility was evaluated by counting the cells that entered capillaries with 0.01 M potassium phosphate buffer (pH 7.0). Bacterial chemotaxis was estimated by counting the cells that entered capillaries filled with a solution of glucose (5.6×10^{-2} M) in the same buffer.

The results of the experiments were statistically processed as described by Lakin [7].

RESULTS AND DISCUSSION

As shown previously, *B. japonicum* cells undergo contact interactions with clay minerals [4]. The present study showed that montmorillonite increased the motility of *B. japonicum* cells in a concentration-dependent manner. For instance, this mineral at concentrations of 0.2 and 1.0 g/l enhanced the motility of *B. japonicum* 634b cells by 25 and 188%, respectively (Fig. 1).

The same effect of the mineral was observed for the highly virulent but non-nitrogen-fixing strain *B. japonicum* 604K. The addition of montmorillonite at concentrations of 0.1 and 0.5 g/l to the cell suspension of this strain augmented cell motility by 27 and 105%, respectively (Fig. 2).

Like montmorillonite, palygorskite increased the motility of the two *B. japonicum* strains, although to a lesser extent. The greatest increase in the motility of *B. japonicum* 634b (by 73%) and 604K (by 28%) was observed at concentrations of palygorskite of 0.5 and 0.2 g/l, respectively (Figs. 3, 4). The effect of palygorskite at concentrations of 0.5 and 1.0 g/l on the motility of *B. japonicum* 604K cells was not as profound as at the concentration of 0.2 g/l.

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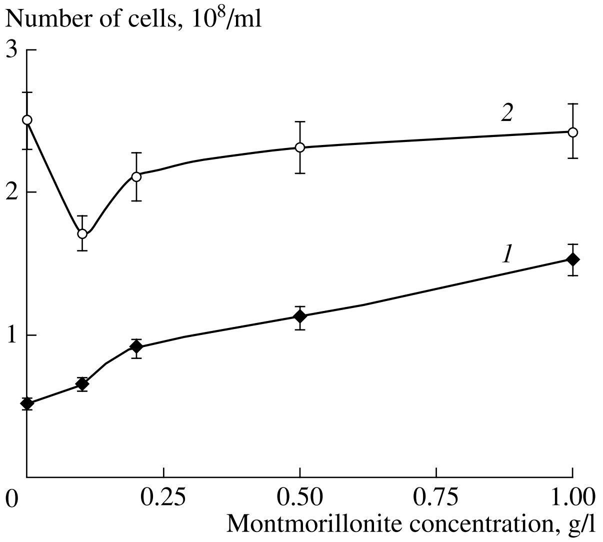

Fig. 1. The effect of different concentrations of montmorillonite on the chemotaxis of *B. japonicum* 634b: the number of cells in capillaries with the potassium phosphate buffer either containing 5.6×10^{-2} M glucose (curve *2*) or not (curve *1*).

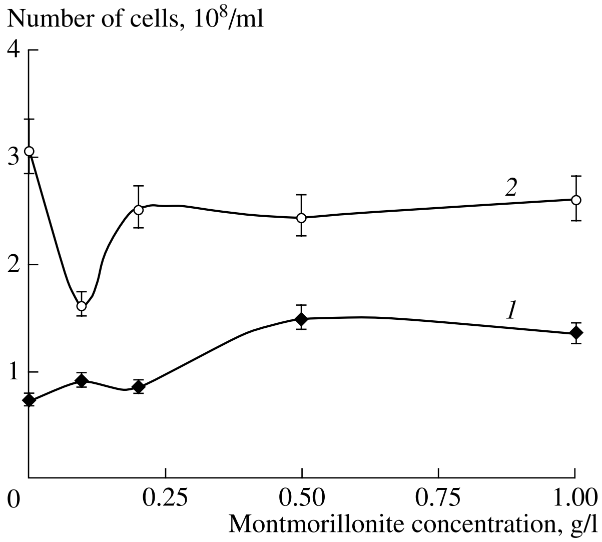

Fig. 2. The effect of different concentrations of montmorillonite on the chemotaxis of *B. japonicum* 604K: the number of cells in capillaries with the potassium phosphate buffer either containing 5.6×10^{-2} M glucose (curve *2*) or not (curve *1*).

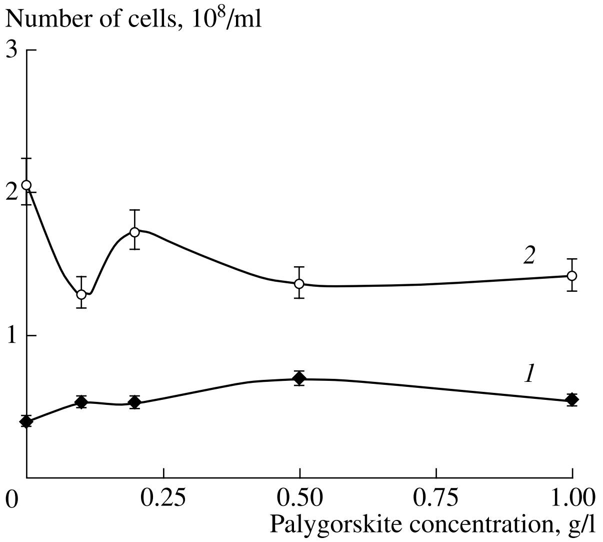

Fig. 3. The effect of different concentrations of palygorskite on the chemotaxis of *B. japonicum* 634b: the number of cells in capillaries with the potassium phosphate buffer either containing 5.6×10^{-2} M glucose (curve *2*) or not (curve *1*).

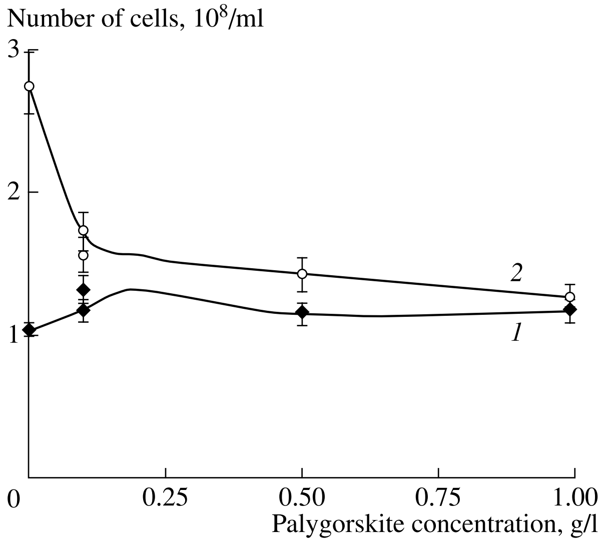

Fig. 4. The effect of different concentrations of palygorskite on the chemotaxis of *B. japonicum* 604K: the number of cells in capillaries with the potassium phosphate buffer either containing 5.6×10^{-2} M glucose (curve *2*) or not (curve *1*).

Thus, the interaction of *B. japonicum* 634b and 604K cells with the clay minerals montmorillonite and palygorskite at concentrations of 0.1 to 1.0 g/l raised cell motility in the absence of attractants.

According to earlier observations [2], these clay minerals enhance the physiological activity of nitrogen-fixing microorganisms, the growth of *B. japonicum* in particular. It is likely that the mechanisms that are responsible for the increase in the physiological activity and the motility of bacterial cells by clay minerals are similar. The possibility cannot be excluded that the effect of montmorillonite and palygorskite on bacterial cells is not limited to the enhancement of their motility. Since clay minerals are constituents of many soil types, their favorable effect on the motility of bradyrhizobia may play a role in the propagation of these bacteria in nature.

At the same time, montmorillonite inhibited the chemotaxis of *B. japonicum* cells, i.e., their directed motion to an attractant (namely, glucose). In particular, at a montmorillonite concentration of 0.1 g/l, the numbers of *B. japonicum* 634b and 604K cells in the capillaries with glucose decreased by 32 and 47%, respectively (Figs. 1, 2). Montmorillonite at higher concentrations (0.2, 0.5, and 1.0 g/l) also diminished the chemotaxis of both bacterial strains.

Similarly to montmorillonite, palygorskite decreased the chemotaxis of both bacterial strains. In particular, palygorskite at a concentration of 0.1 g/l diminished the chemotaxis of *B. japonicum* 634b by 38% (Fig. 3) and to an even greater degree the chemotaxis of *B. japonicum* 604K. All the tested concentrations of the mineral (0.1, 0.2, 0.5, and 1.0 g/l) decreased the number of bacterial cells that entered the capillaries with glucose by 38–54% (Fig. 4).

Thus, the clay minerals decreased the chemotaxis of symbiotically different bacterial strains, the active nitrogen fixer *B. japonicum* 634b and the non-nitrogen-fixing strain *B. japonicum* 604K.

Taking into account that *B. japonicum* cells undergo contact interactions with clay particles, it can be suggested that the decrease in cell chemotaxis may be due to adsorption of these particles on the bacterial surface [4]. This may affect the functioning and eventually cause the blockade of chemotactic receptors, thereby decreasing the chemotaxis of *B. japonicum* 634b and 604K cells.

There may be several mechanisms by which the chemotactic receptors are blocked. For example, the adsorption of fine mineral particles on the bacterial surface may mechanically screen the receptors, or clay particles may come into direct contact with the receptors or some adjacent bacterial surface structures and induce conformational alterations in the receptors, thus causing them to lose the ability to bind attractant molecules.

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