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EXPERIMENTAL ARTICLES

Effect of the Clay Minerals Montmorillonite and Kaolinite on the Genetic Transformation of Competent *Bacillus subtilis* Cells

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Abstract—The effect of the clay minerals montmorillonite and kaolinite on the transformation of competent *Bacillus subtilis* cells with chromosomal DNA was studied. Clay particles were found to substantially increase the transformation frequency of competent cells, as well as the rate of their spontaneous chromosomal and plasmid transformation. The effect was ascribed to the adsorption of bacterial cells on the surface of mineral particles.

Key words: clay mineral particles, competent cells, adsorption, transformation

There is increasing interest in the processes of bacterial gene transfer by genetic transformation, including spontaneous (or natural) transformation. Spontaneous transformation is due to the DNA released into the medium from autolyzed bacterial cells. There are a number of publications dealing with various aspects of the spontaneous transformation of *Bacillus subtilis* and other soil bacteria, some of which are devoted to the preservation of DNA bound to soil particles, clay minerals, etc. [1–3]. It was found that the adsorption of DNA on the clay minerals montmorillonite (M) and kaolinite (K) protects it from degradation by DNase, probably due to the conversion of DNA from the B- to the A-form [4, 5]. To the best of our knowledge, there is no published information as to how the adsorption of competent cells on clay mineral particles may affect their transformation.

The aim of the present work was to approach this problem. In particular, we studied the effect of M and K on the induced and spontaneous transformation of *Bac. subtilis* cells with chromosomal and plasmid DNA.

MATERIALS AND METHODS

Strains and plasmids. *Bacillus subtilis* 168 (p1414), used as the donor of transforming DNA, carried plasmid p1414 cm^r and had the genotype *purB6 leuA8 metB5 thr5* cm^r. Plasmid p1414 cm^r was derived from the cryptic plasmid p1414 by marking it with the chloramphenicol resistance gene cm^r of plasmid pC194 [6]. *Bac subtilis* strain 168, used as the recipient, had the genotype *trpC2 hisB tyrA aroB rif*^{r}. The chromosomal mutation of resistance to 5 μg/ml rifampicin was obtained in our laboratory.

Media. Transformation experiments were carried out using liquid Spitzeisen media 1 and 2 and a solid agar medium based on Spitzeisen medium 2.

Transformation. Procedures for the preparation of competent cells, isolation of transforming DNA, and transformation of bacteria were essentially the same as described in the textbook [7].

Clay minerals. Samples of montmorillonite, fractionated (1μm) Ca-kaolinite, and H-kaolinite were obtained from the Department of Soil Science of the Moscow State University.

RESULTS AND DISCUSSION

Effect of clay minerals present in liquid media on transformation by chromosomal DNA. In the first set of experiments, DNA, at a saturation concentration of 5 μg/ml, was added to a suspension of competent cells in Spitzeisen medium 2 simultaneously with clay minerals (the addition of the minerals was shown not to affect the pH of the medium). All five experiments of this series gave similar results (Fig. 1 presents the data of the typical experiment). It can be seen that M and K added at concentrations of 1–4 mg/ml stimulated transformation, although higher concentrations of these minerals led to a gradual decrease in the number of transformed cells (this range of mineral concentrations is not shown in Fig. 1). The maximum stimulatory effect of M and K (by 4–7 times) was observed in those experiments in which the concentration of transforming DNA was below the saturation level (Table 1).

It was of interest to elucidate whether the observed increase in the transformation frequency was due to DNA adsorption on the surface of minerals or the adsorption of bacterial cells. For this purpose, in the next set of experiments (Table 2 shows the results of the

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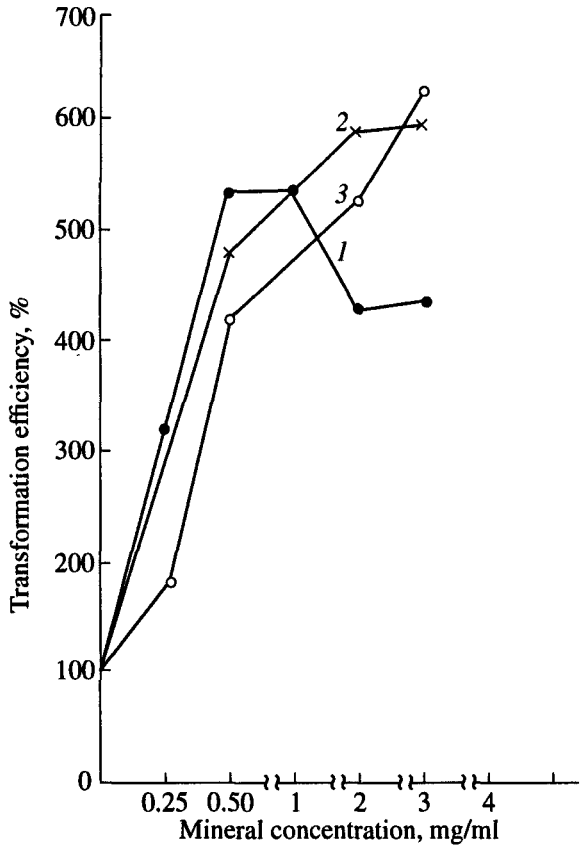

Fig. 1. Effect of (*1*) montmorillonite, (2) H-kaolinite, and (*3*) Ca-kaolinite on the efficiency of chromosomal transformation expressed as the percentage of transformants obtained in the presence of minerals (the number of transformants in the control is taken as 100%).

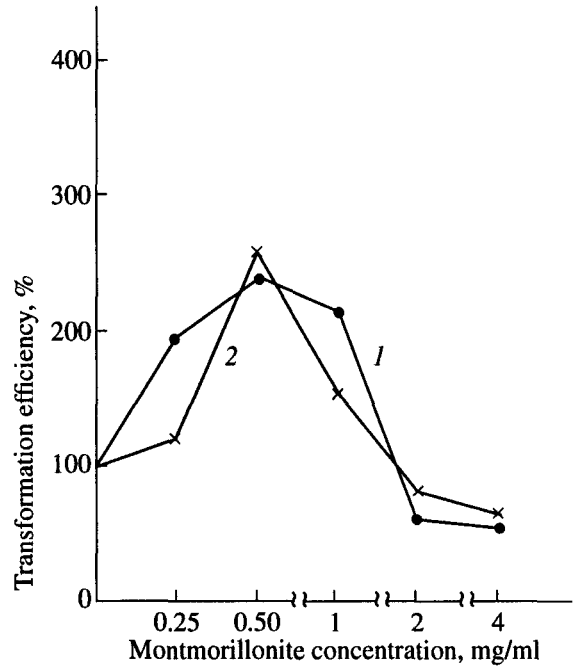

Fig. 2. Effect of montmorillonite on the efficiency of spontaneous (*1*) chromosomal and (*2*) plasmid transformation expressed as the percentage of transformants obtained in the presence of montmorillonite (the number of transformants in the control is taken as 100%).

typical experiment), M particles were preincubated either with competent cells (prior to the addition of DNA) or with DNA (prior to the addition of competent cells).

In the case of mixture 1 (experimental variant with the preincubation of cells with M), *Bac. subtilis* cells cultured in Spitzeisen medium 2 for 1.5 h (until their competence had been reached) were supplemented with M to a final concentration of 1 mg/ml and incubated for 30 min, after which transforming DNA was added. In the case of control mixtures 2 and 3, DNA alone or DNA + M were added to a 2-h culture without the preincubation step. As can be seen from Table 2, the maximum number of transformants (2.8×10^4) were produced in mixture 1, in which competent cells were preincubated with M. This number was 1.5 times greater than in mixture 2, in which mineral particles were added together with DNA without preincubation, and 4 times greater than in mixture 3, in which DNA alone was added to the culture.

In the third set of experiments, we studied the effect of DNA preincubation with M particles on the frequency of cell transformation. DNA was preincubated with M particles for 30 min. In control mixtures 2 and 3, DNA was added to the cell suspension either together with M (but without preincubation) or alone. The results of these experiments are presented in Table 3. It can be seen that the transforming capacity of DNA preincubated with M (mixture 1) was the same as in mixture 2, in which DNA was not preincubated with M particles.

Spontaneous chromosomal and plasmid transformation in the presence of M. As mentioned above, bacterial transformation in natural habitats is due to DNA occurring in the medium as a result of cell autolysis (the so-called spontaneous transformation). It was of interest to evaluate the effect of M on the efficiency of the spontaneous transformation of two types of cells carrying different genetic markers.

In our previous paper [8], we described the strains and selective media that allowed us to select spontaneous transformants resulting from the transfer of the chromosome gene *trpC2* or plasmid p1414 cm^r. The recipient strain required histidine, tryptophan, tyrosine, and phenylalanine for growth and was resistant to rifampicin. The donor strain required adenine, leucine, methionine, and threonine for growth and was resistant, due to the presence of plasmid p1414 cm^r, to chloramphenicol. The genotypes of the recipient and donor strains were such that only the plasmid transformants of the recipient strain could grow on agar media supplemented with histidine, tryptophan, tyrosine, phenylalanine, rifampicin, and chloramphenicol. Donor cells could not grow on this medium, since their require-

Table 1. Effect of Ca-kaolinite on the genetic transformation frequency

Number of transformants in 1 ml	DNA concentration, μg/ml		
	0.4	0.8	2.0
In the presence of 2 mg/ml Ca-K	175	560	1610
Control (without Ca-K)	25	400	1230

ments for adenine, leucine, methionine, and threonine were not satisfied, and they were inhibited by rifampicin. The reverse transfer of DNA genes from the recipient to the donor strain was, in fact, possible. However, a simultaneous transfer of five genes to the donor strain to make it autotrophic and resistant to rifampicin was impossible (this was confirmed in experiments in which we attempted to transform donor cells with the DNA isolated from recipient cells). To select the spontaneous transformants of recipient cells with the chromosome marker *trpC2*, the selective medium was supplemented with histidine, tyrosine, phenylalanine, and rifampicin, but not with tryptophan.

In this set of experiments, donor and recipient strains were grown in Spitzeisen medium 1 to the late exponential phase, and the 0.5-ml aliquots of each strain were added to tubes with 5 ml of Spitzeisen medium 2 containing various amounts of M. The tubes were incubated for 3 h, and then mixed cultures were plated onto selective media to detect the plasmid or chromosomal transformants of the recipient strain.

The results of one of the seven experiments of this series are presented in Fig. 2. In this experiment, M stimulated both chromosomal and plasmid transformation by 2–2.5 times (in some experiments, M increased the transformation rate by about a factor of ten). The maximum effect of M was observed when the concentration of this mineral was 0.5 mg/ml.

The results reported here suggest that the stimulation of *Bac. subtilis* transformation by minerals is due to the adsorption of bacteria on mineral particles, since the preincubation of DNA with M did not increase the transformation rate (probably, the decrease in the susceptibility of DNA to nucleases due to adsorption on the surface of minerals was insignificant). These results are in good agreement with the data of Lorenz *et al.* [9], who showed that the genetic transformation of *Bac. subtilis* cells attached to sand grains was 25 to 50 times more efficient than in the liquid phase. Some discrepancy between our results, which indicate a better transformation of attached cells at a normal transformation temperature (37°C), and those obtained by Lorenz *et al.*, which indicate that attached cells are transformed better than free cells only at 23°C, can be explained by the low efficiency of transformation at this temperature (about 4% of the transformation efficiency at 37°C).

The mechanism of this phenomenon remains unknown, although it can be anticipated that the simul-

Table 2. Effect of the preincubation of competent cells with montmorillonite on the genetic transformation frequency

Experimental protocol				Number of transformants in 1 ml
Mixture 1:		Addition of DNA		
Suspension of competent cells with montmorillonite				2.8×10^4
Mixture 2:	30-min incubation	Addition of DNA and montmorillonite	30-min incubation and plating	
Competent cells				1.8×10^4
Mixture 3:		Addition of DNA		
Competent cells				0.7×10^4

Table 3. Effect of the preincubation of DNA with montmorillonite on the genetic transformation frequency

Experimental protocol				Number of transformants in 1 ml
Mixture 1:		Addition of competent cells		
Suspension of DNA and montmorillonite				5.8×10^3
Mixture 2:	30-min incubation	Addition of competent cells and montmorillonite	30-min incubation and plating	
DNA				6.0×10^3
Mixture 3:		Addition of competent cells		
DNA				1.7×10^3

taneous adsorption of competent recipient cells and DNA molecules on clay particles increases the probability of DNA penetration into cells, or that the adsorption of cells on clay particles enhances their competence. Indeed, it is known that the adsorption of bacterial cells on solid surfaces can substantially affect their physicochemical properties [10], and that conjugation (another type of genetic transfer) is enhanced when bacterial cells are adsorbed on the surface of submerged plants, stones, and sewage pipes, forming biofilms [11].

The results presented in this paper also indicate that the adsorption of soil bacilli on M and K particles in natural habitats can facilitate the transfer of DNA from one cell to another. Furthermore, we use the phenomenon of the increased efficiency of transformation on clay mineral in our laboratory practice to augment the number of chromosomal and plasmid transformants, especially when only very low concentrations of DNA are available.

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