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EXPERIMENTAL ARTICLES

The Electrooptical Parameters of Suspensions of *Escherichia coli* XL-1 Cells Interacting with Helper Phage M13K07

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Abstract—The electrophysical properties of *Escherichia coli* XL-1 cells interacting with helper phage M13K07 were studied as a function of the phage-to-cell ratio and the contact time. The electro-optical signal of bacterial cells changed considerably as soon as 10 min after the onset of their incubation with phage particles, presumably due to phage adsorption on the cell surface. The maximum changes in the orientational spectra of cell suspensions were observed when the phage-to-cell ratio was 20. Selectivity studies showed that *E. coli* XL-1 cells interacting with the helper phage M13K07 in the presence of foreign microflora, such as *E. coli* K-12 or *Azospirillum brasilense* Sp7, can be identified by using their electrophysical properties. Changes in the orientational spectra of cell suspensions are interpreted with the stage of phage–bacterium interaction taken into account. The results obtained can probably be used to devise a new rapid method for identification of microorganisms and to study the particular stages of cell infection by bacteriophages.

Key words: *Escherichia coli* XL-1, electrooptical spectrum, helper phage M13K07, transfection, bacterium-phage binding.

There is increasing interest among researchers in the study of the electrophysical properties of microbial cells. This is due to the existence of a relationship between the electrophysical properties of microbial cells and their morphology and surface structure that makes possible a nondestructive study of these cells [1, 2]. One of the most promising relevant electrophysical methods (the electroorientation of cells in alternating electric fields), which also has long been known, received an impetus from the recent manufacture of an original electro-optical analyzer, ELUS. This analyzer makes it possible to perform measurements by changing the orienting electric field parameters, such as frequency, intensity, and switch-on time. Our recent studies showed that ELUS can be efficiently used for investigating the heterogeneity of cell suspensions [3, 4] and the effect of low-molecular-weight toxic compounds on microbial cells [5–7]. Along with the well-known microbiological, biochemical, immunological, and gene engineering approaches, electrophysical analysis can presumably be used for the taxonomic identification of bacteria.

It should be noted that microorganisms can also be identified with the use of specific bacteriophages [8, 9], which allow the species and type affiliation of bacteria to be easily performed [10, 11]. The interaction of bacterial cells with phages is a complex process, which depends on the phage type (temperate, virulent, etc.) [12–15]. In particular, virulent phages can cause lytic reactions and cell lysis from outside, whereas temperate phages make host bacterial cell lysogenic.

The aim of this work was to study the electrophysical properties of *Escherichia coli* XL-1 cells interacting with moderate filamentous bacteriophage M13K07, which specifically infects male (containing F pili) *E. coli* cells without causing their lysis [16, 17]. The filamentous phage infection [14, 15] begins with the interaction of the minor phage capside protein G3P (gene 3 protein) with bacterial F pili (which are the primary phage receptors) and then with the integral membrane protein TolA [15]. The penetration of phage DNA to the cytoplasm of *E. coli* cells and the incorporation

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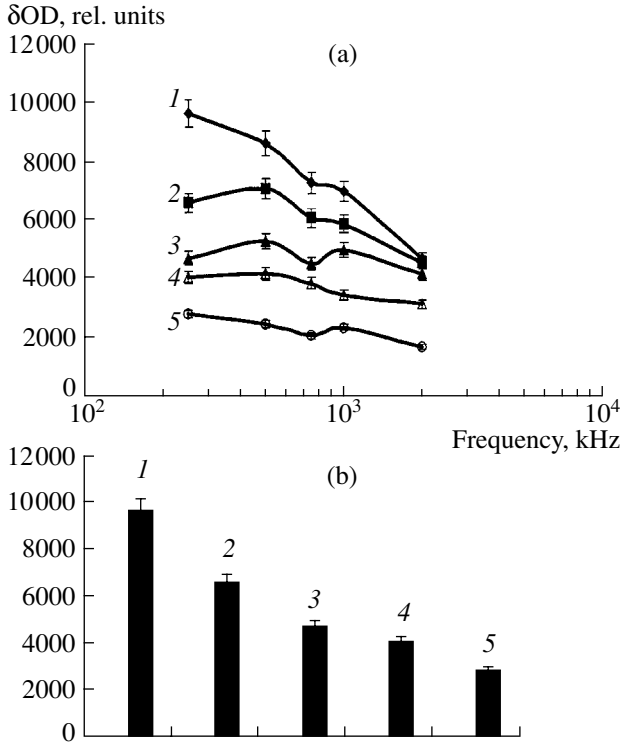

Fig. 1. The effect of the phage M13K07 on (a) the orientational spectra and (b) changes in the relative parameters of the suspension of *E. coli* XL-1 cells in distilled water with a conductivity of 1.8 μS/cm. For purposes of simplifying the figure, it shows only the spectra recorded in the orienting electric field with a frequency of 250 kHz. The phage was added in the proportion of (*1*) 0, (*2*) 1, (*3*) 5, (*4*) 10, and (*5*) 20 particles per bacterial cell.

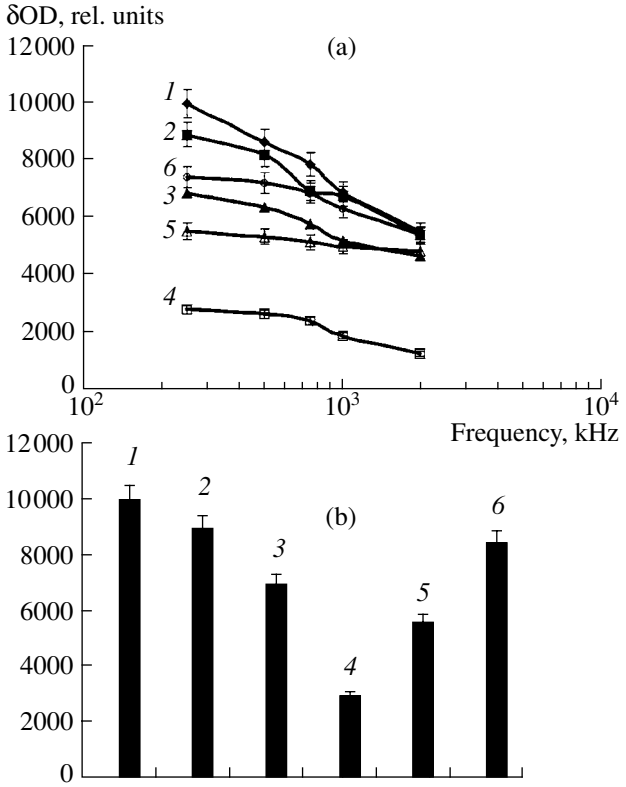

Fig. 2. (a) The orientational spectra and (b) changes in the relative parameters of the suspension of *E. coli* XL-1 cells in distilled water with a conductivity of 1.8 μS/cm. The M13K07 phage was added in the proportion of 20 particles per bacterial cell. For purposes of simplifying the figure, it shows only the spectra recorded in the orienting electric field with a frequency of 250 kHz. The spectra were recorded after (*1*) 0, (*2*) 1, (*3*) 10, (*4*) 30, (*5*) 60, and (*6*) 90 min of cell contact with the phage.

of the G8P capside protein into the inner cytoplasmic membrane bring about considerable changes in the properties of the infected microbial cells and presumably must influence the electrophysical parameters of the cell suspensions.

MATERIALS AND METHODS

Experiments were carried out with two *Escherichia coli* strains, XL-1 and K-12, and *Azospirillum brasilense* Sp7, which were obtained from the collection at the Institute of Biochemistry and Physiology of Plants and Microorganisms. The strains were grown aerobically at 30°C on a shaker (160 rpm) in a liquid nutrient medium containing (g/l) NaCl, 10; yeast extract, 5; and peptone, 5. Bacterial cells grown in this medium for 1 day were harvested and used for electro-optical measurements.

Cell preparation. Before measurements, cells were washed three times by centrifugation at 2800 *g* for 5 min and resuspended in a small volume of distilled water. The cell suspension was centrifuged at 110 *g* for 1 min to remove cell clumps, and the supernatant was used for electro-optical measurements.

The orientational spectra of bacterial cells were recorded at 670 nm [6] by using an ELBIC electro-optical analyzer designed and manufactured at the State Research Center for Applied Microbiology, Obolensk, Moscow oblast. The orienting electric field had discrete frequencies of 250, 500, 750, 1000, and 2000 kHz. The orientational spectrum of a cell suspension represented the frequency dependence of the difference of the optical densities of the cell suspension measured along and across the orienting field vector. The optical density difference was normalized to the optical density of the cell suspension measured at a random cell orientation. There are grounds to believe that, at certain wavelengths of the light beam and the amplitude of the orienting electric field, the orientational spectrum is mainly determined by the frequency-dependent cell anisotropy [1, 3].

Cell infection was carried out with the kanamycin-resistant filamentous phage M13K07 (Stratagene, Sweden) from the *Inoviridae* family, which was derived from the wild phage M13 [18, 19]. For infection, *E. coli* XL-1 cells were transferred from a single colony grown

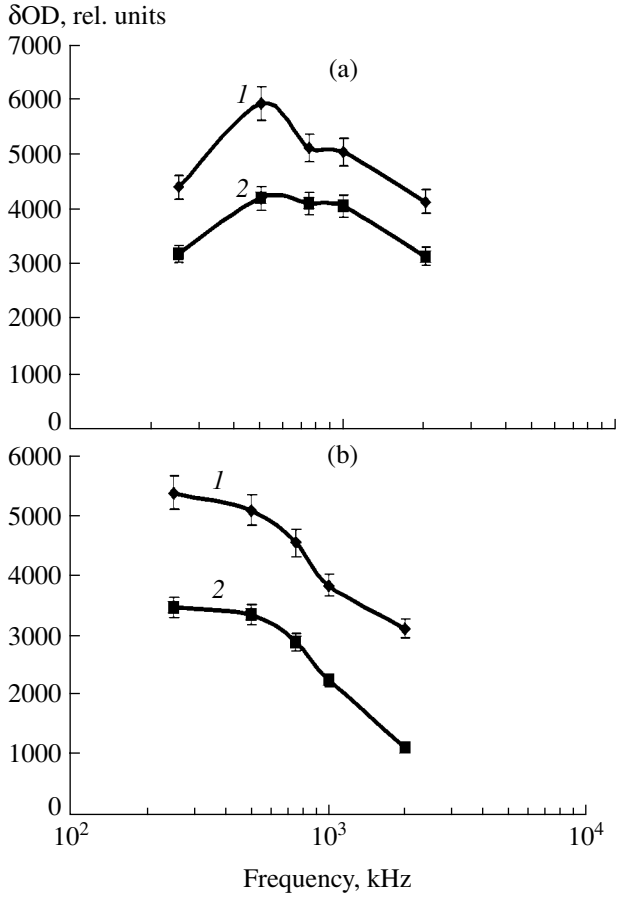

Fig. 3. The effect of the phage M13K07 on the orientational spectra of the mixed suspensions of (a) *E. coli* XL-1 and *E. coli* K-12 cells and (b) *E. coli* XL-1 and *A. brasilense* Sp7 cells in distilled water with a conductivity of 1.8 μS/cm. The M13K07 phage was added in the proportion of (*1*) 0 and (*2*) 20 particles per bacterial cell. For purposes of simplifying the figure, it shows only the spectra recorded in the orienting electric field with a frequency of 250 kHz.

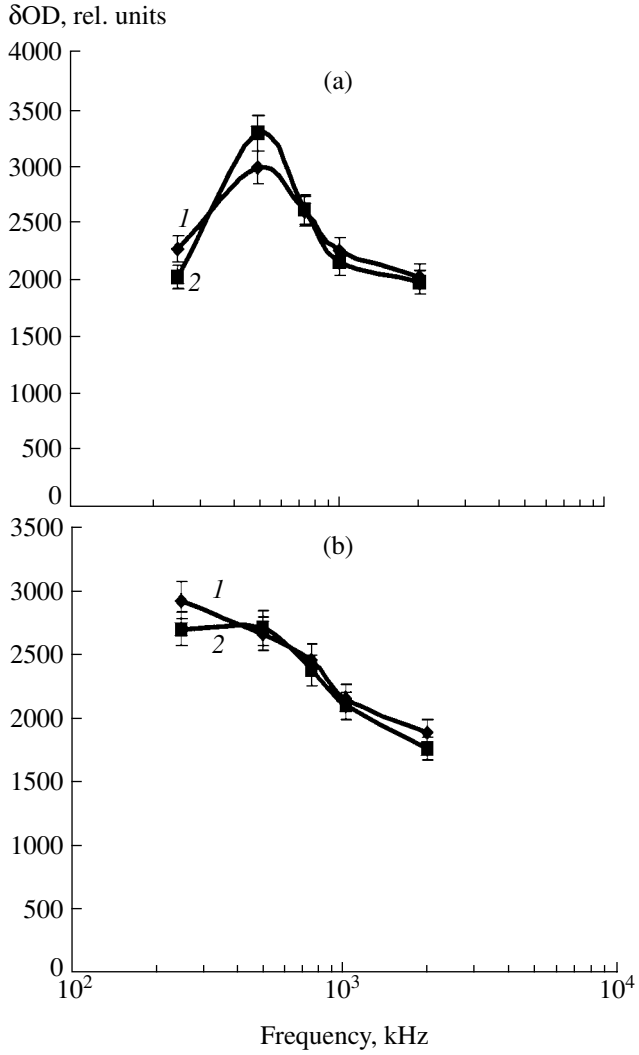

Fig. 4. The effect of the phage M13K07 on the orientational spectra of the phage-resistant (a) *E. coli* K-12 and (b) *A. brasilense* Sp7 cells in distilled water with a conductivity of 1.8 μS/cm. The M13K07 phage was added in the proportion of (*1*) 0 and (*2*) 20 particles per bacterial cell. For purposes of simplifying the figure, it shows only the spectra recorded in the orienting electric field with a frequency of 250 kHz.

on a plate with LB agar containing 12.5 μg/ml tetracycline to 2 ml of liquid LB broth and cultivated overnight at 37°C with continuous aeration. One-tenth of the overnight culture was transferred to fresh medium and cultivated at 37°C to the early exponential growth phase (D_{600} = 0.5–0.6, which corresponded to a culture density of 7×10^8 cells/ml) [19]. At this time, the aeration of the culture was ceased for 30–40 min to restore F pili, which are involved in the infection of bacterial cells with bacteriophages. Then the culture was mixed with a suspension of the M13K07 phage particles, the number of which exceeded the number of bacterial cells by 20 times. The mixture was incubated at 37°C for 1, 10, 30, 60, and 90 min under stationary conditions (to induce the sorption of the phage particles on the pilus surface). After such incubation, the cell suspension was subjected to electro-optical measurements.

RESULTS AND DISCUSSION

Experiments were carried out with the helper phage M13K07 and *E. coli* XL-1 cells, which carry F episome and produce F pili, due to which the cells can be infected with the phage [19].

Changes in the electro-optical parameters of the *E. coli* XL-1 cells were studied as a function of the relative number of phage particles added to the cell suspension. The orienting electric field had discrete frequencies of 250, 500, 750, 1000, and 2000 kHz; an intensity of 17 V/cm; and turn-on time of 16 s. The number of bacterial cells in the suspension was 7×10^8 cells/ml. The cells were cultivated and all the measurements were carried out at a temperature of 37°C,

which is beneficial for the formation of F pili on the surface of bacterial cells. The orientational spectra of the cell suspension were recorded 30 minutes after the mixing of bacterial cells and phage particles. The maximum changes in these spectra were observed when the relative number of phage particles was 20 per microbial cell (Fig. 1a). All further experiments were performed by mixing cells and phage particles in this proportion. To control the process of infection, bacterial cells were cultivated in LB broth with kanamycin, since phage M13K07 is resistant to this antibiotic [18]. The good growth of bacterial cells in the presence of kanamycin indicated that they were infected with the phage.

The study of the effect of different incubation times (1, 10, 30, 60, and 90 min) on the electro-optical signal of bacterial cells showed that as soon as after 5-min incubation, phage particles induced a noticeable decrease in the electro-optical signal of these cells (Fig. 2). The decrease in the electro-optical signal reached a maximum after 30 min of cell incubation with the phage. However, after the next 30 min of incubation, the electro-optical signal of the cell suspension increased again (Fig. 2).

Further experiments were carried out with *E. coli* XL-1 cells mixed with foreign microflora, namely, *E. coli* K-12 (which is the parent strain for XL-1 and, hence, must be susceptible to the phage M13K07) and *A. brasilense* Sp7 (this strain is taxonomically different from XL-1 but has the same size of cells). Phage particles were added to the mixed suspensions (OD_{665} = 0.42–0.44) of *E. coli* XL-1 and K-12 cells (Fig. 3a) and *E. coli* XL-1 and *A. brasilense* Sp7 cells (Fig. 3b) in the same proportion of 20 particles per bacterial cell as in the previous experiments with the monosuspension, all other experimental conditions being also the same. These experiments showed that the foreign microflora considerably diminished the electro-optical signal of the *E. coli* XL-1 cells (Fig. 3). The control experiments showed that neither the electro-optical signal of *E. coli* K-12 cells (Fig. 4a) nor the electro-optical signal of *A. brasilense* Sp7 cells (Fig. 4b) changed in response to the addition of the phage in the proportion of 20 particles per bacterial cell. The resistance of the *E. coli* K-12 and *A. brasilense* Sp7 cells to infection by the phage M13K07 was confirmed by the standard microbiological approach.

Thus, the M13K07 phage influenced only the electro-optical parameters of the susceptible *E. coli* XL-1 cells and did not influence the electro-optical parameters of the *E. coli* K-12 and *A. brasilense* Sp7 cells, which are resistant to this phage. The presence of the *E. coli* K-12 and *A. brasilense* Sp7 cells in the mixed bacterial suspension did not prevent the specific action of the phage M13K07 on the electro-optical signal of the *E. coli* XL-1 cells. The change in the electro-optical signal of the *E. coli* XL-1 cells in response to the addition of the phage particles depended on the relative number of these particles and the contact time. All this suggests that electro-optical analysis can be used to study the stages of bacterial infection with bacteriophages, among which the M13K07 phage is of particular interest as being symbiotic and unable to lyse the bacterial cells. We believe that the data obtained can be useful in devising a new rapid method for identification of microorganisms.

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