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EXPERIMENTAL ARTICLES

Effect of Growth Conditions on Electrophysical Properties of *Rhodobacter capsulatus* PG Cells

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Abstract—Electrophysical characteristics of cells of the phototrophic bacterium *Rhodobacter capsulatus* PG grown in complete Hutner medium in light or dark were found to differ depending on the composition of their lipopolysaccharides (LPS). Under dark cultivation, the cells synthesized LPS with a shortened structure that determined the electrophoretic properties of cell surfaces. The observed decrease in the effective high-frequency electroconductivity of the dark-grown cells is assumed to be due to a decrease in the intracellular K^+ concentration resulting from increased permeability of cytoplasmic membranes of the cells grown under these conditions.

Key words: Rhodobacter capsulatus, growth conditions, lipopolysaccharide, electrophysical properties.
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In the representatives of the genus *Rhodobacter*, the organization of the cell wall is typical of gram-negative bacteria, with lipopolysaccharides (LPSes), which occupy two thirds of the cell surface, peptidoglycan, lipoproteins, porins, and other proteins and phospholipids as its macromolecular components [1].

The representatives of the genus *Rhodobacter* are known to respond to changes in growth conditions by switching between different metabolic pathways involved in energy generation [2, 3].

Earlier, we have observed a change in the molar ratio between lipid A and the polysaccharide fragment of the LPS molecule in the cells of *Rb. capsulatus* PG grown in complete Hutner medium in the dark [4]. According to literature data, the LPS structure in enterobacteria determines the surface properties of the cells to a considerable degree. This conclusion follows from the resemblance between the values of the electrokinetic potential (EKP) of the LPS preparations and the original EKP values of the cells from which these preparations were isolated [5]. We have demonstrated that both the LPS structure and the chemotype of phototrophic bacteria affected the surface electric properties of the cells [6].

The method of electroorientational spectroscopy (EO-spectroscopy) is widely used to study the electrophysical characteristics of cytoplasmic membranes and cytoplasm of living bacterial cells [7]. However, electrophysical properties of phototrophic bacteria are poorly known.

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The method of cell electrophoresis is most frequently used for the study of the cell surface; it enables very accurate characterization of the cell–environment interface [7]. EKP is a calculated value, which is obtained from the measurements of electrophoretic mobility (EPM) of the cells. The LPS molecules of the outer layer of the cell wall are not homogeneous due to the peculiarities of their biosynthesis; they constitute a family of molecules, which represent different stages of biosynthesis and differ in their structure and molecular weight [8]. Such heterogeneity is revealed by electrophoresis of the LPS preparations. In the literature, the opinion exists that the pattern of electrophoresis of LPS from *Enterobacteriaceae* is a "fingerprint" for bacteria at the strain level [9].

The aim of the present work was to study the effect of light and dark cultivation of the phototrophic bacterium *Rb. capsulatus* PG on the LPS composition of the bacterial cell wall and electrophysical characteristics of the cells.

MATERIALS AND METHODS

The purple nonsulfur phototrophic bacterium *Rhodobacter capsulatus* PG VKM B-2381D obtained from the VKM (All-Russian Collection of Microorganisms) was grown anaerobically in liquid Hutner medium [10] at 30°C and 1000–2000 lx illumination for 4–5 days. The purity of the culture was tested routinely by plating [11].

A five-day culture of *Rb. capsulatus* PG grown anaerobically in light under optimal conditions was

inoculated (20–25% of the total medium volume) into complete Hutner medium and grown in light or dark. Sampling was performed after five days of cultivation.

The isolation of LPS from the *Rb. capsulatus* cells was carried out by Westphal's method with the modification of Kul'shin et al. [12].

The LPS were characterized by the method of vertical electrophoresis in 14% polyacrylamide gel (PAG) according to Krauss [13]. The thickness of the gel was 1 mm; the LPS amount per track was 5 μg.

Electroorientational spectra (EO spectra) of the cells were obtained by measuring relative changes in the optical density of the cell suspensions resulting from the cell alignment in a uniform alternating electric field of a certain frequency *f* ranging from 0.5 to 10 MHz (at field intensity of 60 V/cm) at 20°C [7]. Before measurements, the cells were twice washed with double distilled water and resuspended in Tris–HCl buffer (specific electric conductivity of 3.7×10^{-3} S/m; pH 7.0) up to the concentration of 8×10^7 cells/ml.

For electrophoretic measurements, the cells were twice washed with phosphate–citrate buffer with ionic force of 0.02 and pH 7.0 and precipitated by centrifugation; the cell suspensions (5×10^6 cells/ml) were prepared in the same buffer. The EPM of 25 cells was determined with a Parmoquant-2 microscope (Carl Zeiss, Jena, Germany) at 20°C. The mean EPM value, the standard deviation, and the mean arithmetic error were determined for each sample. The reliability of the results was calculated using the Student–Fisher criterion. The results represent the mean values of triplicate experiments.

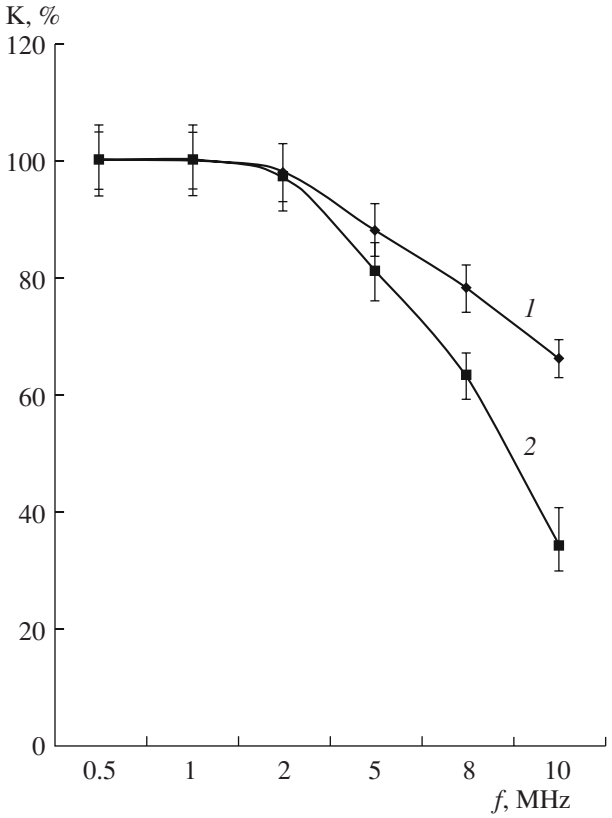

Fig. 1. Electroorientational spectra of intact cells of *Rb. capsulatus* PG cultivated in light (*1*) or dark (*2*). The assay medium is Tris–HCl buffer; specific conductivity 3.7×10^{-3} S/m; pH 7.0. $K = \Delta D_i/\Delta D_o \times 100\%$, where ΔD_i and ΔD_o signify a change in the optical density of cell suspensions after switching on the electric field and at the field frequency of 0.5 MHz, respectively.

RESULTS AND DISCUSSION

Rhodobacter are asporogenic bacteria with rod-shaped or oval cells, 3–5 μm in length and 0.5–1.2 μm in diameter. Cells multiply by binary division; capsules and mucus can be produced; chains of cells can be formed [14].

According to literature data, the maximal growth rate of *Rb. capsulatus* was observed under photoheterotrophic anaerobic conditions [15]. The bacteria can also grow anaerobically in the dark in the presence of glucose or other organic compounds, switching from photosynthesis to energy generation through fermentation or anaerobic respiration [2]. *Rb. capsulatus* can also grow in the dark under microaerobic conditions and oxidize molecular hydrogen [3].

High-frequency EO spectra of the dark- and light-grown *Rb. capsulatus* PG cells are given in Fig. 1. A decline in the EO spectra of the dark-grown cells was shifted towards a region of lower frequencies as compared with that of the light-grown cells. A high-frequency decline of the EO-spectra is known to be determined mainly by the effective specific electric conductivity of the cytoplasm, which, in turn, depends on the amount and mobility of intracellular ions, mainly K^+. A comparison of experimental data on the high-frequency decline of the EO spectra of the cells grown under different illumination conditions with the results of theoretical analysis of the cell electroorientation revealed that the values of the effective high-frequency conductivity of the light- and dark-grown cells were about 0.5 ± 0.08 and 0.32 ± 0.06 S/m, respectively [16]. Our results are in good agreement with the data obtained for other microbial species [17]. The comparatively low value of the effective high-frequency conductivity of dark-grown cells can be due to a decrease in the intracellular concentration of K^+ ions because of increased permeability of the cytoplasmic membranes of bacteria cultivated under these conditions. A direct measurement of intracellular concentration of K^+ in *Rb. capsulatus* PG grown under different illumination conditions could help to confirm our assumption.

Electrophoretic properties of bacteria are known to depend on the structure of the cell wall LPS [5]. That is why we performed comparative studies of the LPS preparations from the light- and dark-grown cells of *Rb. capsulatus* PG by electrophoresis in 14% PAG. As seen from Fig. 2, the LPS preparations from the dark-grown

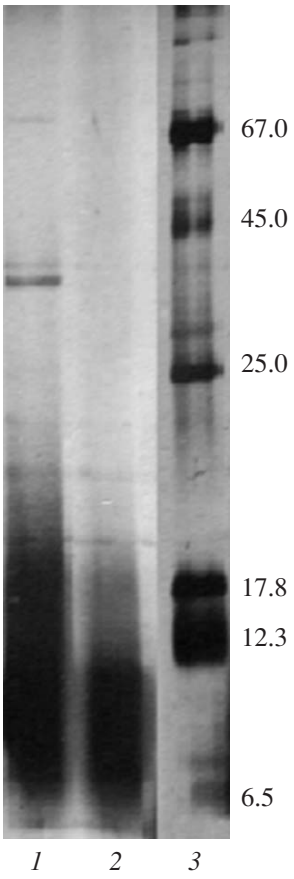

Fig. 2. Electrophoregram of LPSes isolated from light-grown (*1*) and dark-grown (*2*) cells of *Rb. capsulatus* PG. Marker proteins (*3*). The molecular weights of marker proteins in kDa are given in the right column.

cells contained no colored bands in the high-molecular-mass region. This is an indication of synthesis of polysaccharide fragments with incomplete structure under dark conditions because of limitation or alteration of its biosynthesis [8]. According to Krauss, the LPS from light- and dark-grown cells can be connected to the SR and R chemotypes, respectively [13].

The dark- and light-grown cells differed not only in the composition of their LPS determined by electrophoresis in 14% PAG, but also in the electric properties of their surfaces. The results of the study of electrophoretic properties of the dark- and light-grown cells of *Rb. capsulatus* PG are presented as the mean values of the cell EPM (table) and in the form of histograms of

The mean values of EPM of the *Rb. capsulatus* PG cells cultivated in light or dark

Growth conditions	EPM, μm S^{-1} V^{-1} cm	Significance level *P*
Light	–1.92 ± 0.015	
Dark	–2.03 ± 0.03	$P < 0.001$

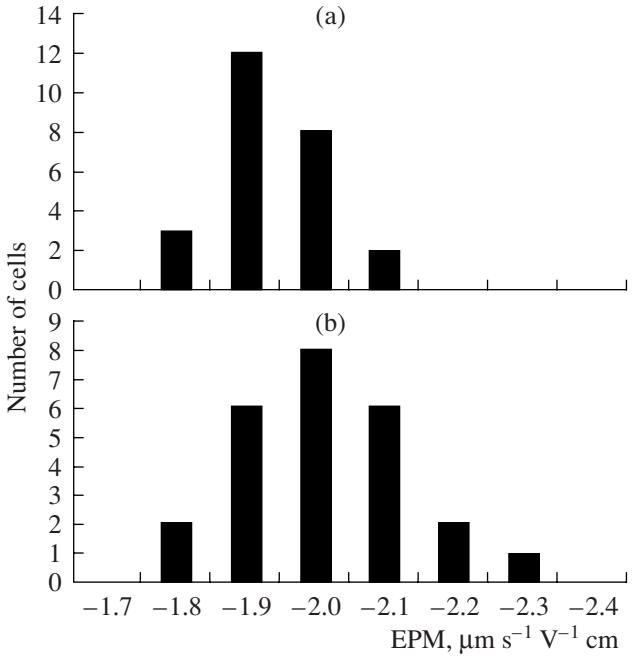

Fig. 3. Histograms of the EPM distribution in the cells of *Rb. capsulatus* PG cultivated in light (a) and dark (b). The assay medium is phosphate–citrate buffer (pH 7.0) with ionic force of 0.02; specific conductivity is 1.4 mS/cm.

the EPM distribution in the cells from different populations (Fig. 3).

Electrokinetic measurements revealed that the mean negative EPM value of dark-grown cells of *Rb. capsulatus* PG was reliably higher than that of the light-grown cells, indicating the synthesis of LPS with a shortened structure under dark conditions. These results are in agreement with our data on electrophoretic properties of the LPS preparations. Moreover, the dark cultivation of bacteria resulted in increased heterogeneity of the EPM values in the cell population. The presence of polysaccharides in the LPS of the cell surface of the light-grown *Rb. capsulatus* PG seemed to screen the main (negative) groups in the core and/or shift the electrokinetic sliding surface from the charged cell surface, thus resulting in a lower negative value of EPM of the cells from this population. Higher heterogeneity of the EPM values in the population of the dark-grown cells is apparently caused by the presence of the LPS molecules with different lengths of the polysaccharide fragments at the cell surface. The observed effect of the LPS composition on the surface electric properties of *Rb. capsulatus* PG cells conform to our earlier data on the correlation between a chemotype of phototrophic bacteria of the genus *Rhodobacter* and electrophoretic properties of the cells [6].

Thus, it was shown that the cells of *Rb. capsulatus* PG grown in complete Hutner medium in dark or light differed in the composition of their LPS. The dark-grown bacteria produced LPS with a shortened struc-

ture that affected electrophoretic properties of their cell surfaces. Moreover, cell cultivation in the dark caused a shift in the cell metabolism that resulted in a decrease in the effective high-frequency conductivity of the cells, probably due to a change in intracellular concentration of K^+ ions. These results make it possible to predict the behavior and properties of bacteria cultivated under different conditions.

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