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EXPERIMENTAL ARTICLES

Relations between the Chemotype of *Rhodobacter capsulatus* Strains and the Cell Electrophoretic Properties

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Abstract—The cells of two *Rhodobacter capsulatus* strains, B10 and PG, and the LPS of their cell walls were studied by electrophysical and biochemical methods. Strain B10 was found to belong to the R chemotype, and strain PG, to the RS chemotype. A relation was revealed between the chemotype of the photosynthesizing bacteria *Rhodobacter capsulatus* and the electrophoretic properties of their cells.

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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 (LPS), which occupy two thirds of the cellular surface; peptidoglycan of the A1γ-type; lipoproteins; porins and other proteins; and phospholipids as its macromolecular components [1].

In the course of division or death of bacteria, LPS are liberated in the form of free molecules or complexes with proteins; they exhibit various forms of biological activity and participate in the pathogenesis of septic shock, an important cause of mortality throughout the world [2].

LPS activate the production of different cytokines (in particular, TNF-α, IL-1, and IL-6) by blood cells; these compounds, in turn, act as endogenous mediators of inflammation [3]. By inhibiting apoptosis of the neutrophiles circulating in the bloodstream, the endotoxins cause an increase in their lifespan [4].

Gram-negative bacteria belonging to the S chemotype form smooth colonies on solid media, while the cells of R chemotype form rough ones. The complete molecule of an S-LPS consists of lipid A, an oligosaccharide core (which is subdivided into internal and external ones), and the O polysaccharide (O antigen). In a molecule of an R-LPS, the polysaccharide fragment is absent. The bacterial chemotype is determined by the structure of the LPS contained in the bacterial cell wall.

The structure of enterobacterial LPS determines the surface properties of the cells to a considerable extent; this is evident from 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]. The method of cell electrophoresis is most frequently used for the study of cell surfaces; it enables very accurate characterization of the cell–environment interface [6]. EKP is a calculated value which is obtained from the measurements of electrophoresis mobility (EPM) of the cells. The study of EKP of *Escherichia coli* and *Salmonella* bacterial cells of different chemotypes revealed that the maximum EKP values were observed in deep Re-Rd mutants, which contain in the core only 2-keto-3-deoxyoctolusonic acid (KDO) residua and a heptose part. This result can be explained by the localization of the major negatively charged groups (KDO, phosphates) in the internal core of the region. The smallest EKP values are characteristic of the S chemotype cells; they carry complete O-polysaccharide chains, which screen the surface charge. Another explanation for the difference in the charges of individual strains can be related to the presence of other noncarbohydrate substitutes in the LPS molecule, which provide its specific charge (phosphates, ethanolamine, amine) [5].

LPS 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 [7]. This heterogeneity is revealed by electrophoresis of LPS preparations. In the literature, the opinion exists that the picture of *Enterobacteriaceae* LPS electrophoresis separation is a "fingerprint" for bacteria at the strain level [8]. Comparative electrophoretic analysis of the LPS isolated from different strains of *Rhodobacter capsulatus* and *Rb. sphaeroides* confirmed the validity of this

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assertion for the LPS of photosynthetic bacteria [9]. The picture of the distribution of the LPS zones in the gel reflects the characteristics of lipopolysaccharide biosynthesis not only for the bacterial species, but for the strain as well [10]. Thus, if agglutination by the Lindberg method [11] permits relatively accurate determination of the S or R chemotype of bacteria, the picture of LPS electrophoretic distribution can serve as a "fingerprint" for the strains of photosynthetic bacteria with an uncharacterized core structure, thus providing much more complete information about the LPS structure.

The determination of a bacterial chemotype is achieved by a complex of methods, including genetic engineering, phage typing, serology, determination of LPS composition and structure, and the study of the combination of its biological and physicochemical properties.

The goal of this work was to study the chemotypes of two strains of the photosynthesizing bacterium *Rb. capsulatus* B10 and PG, as well as their relation to the electrophoretic characteristics of cells.

MATERIALS AND METHODS

The cultures of purple nonsulfur photosynthetic bacteria *Rhodobacter. capsulatus* B10 from the collection of the Department of Microbiology, Moscow State University, and *Rb. capsulatus* PG VKM V-2381D from VKM, Russian Academy of Sciences, were the objects of our study. The cells of strains B10 and PG were grown for 4–5 days on Hunter medium [12] under anaerobic conditions at 30°C and 1000–2000 lx illumination in liquid culture and on solid medium with 1.5% agar.

The registration of the absorption spectra of the cell suspensions was performed relative to control (growth medium) with a Hitachi 557 spectrophotometer (Japan) in the wavelength range from 300 to 900 nm.

Cell agglutination was performed according to Lindberg [11].

For the electrophoretic measurements, the cells were twice washed with phosphate–citrate buffer with ionic force of 0.02 and pH 7.0 [13] and precipitated by centrifugation; cell suspensions with the required pH value and the concentration of 5×10^6 cells/ml were prepared. The EPM of 25 cells was determined with a Parmoquant microscope (Carl Zeiss, Jena, Germany) at 20°C. The average EPM, the standard deviation, and the mean arithmetic error were determined for each test. The reliability of the results was determined by the Student–Fisher criterion.

LPS isolation from *Rb. capsulatus* cells was performed by Westfal's method in the modification of Kul'shin et al. [14]. LPS content in the supernatants after centrifugation of cell suspensions was determined by the spectroscopic method with a carbocyanine dye [15]. Absorption spectra of the LPS–carbocyanine complex were recorded in the wavelength range from 450 to 478 nm with a Hitachi spectrophotometer (Japan).

The KDO content in the LPS preparations was determined by the reaction with 2-tiobarbituric acid [16].

LPS were characterized by the method of vertical electrophoresis in 14% polyacrylamide gel (PAAG) according to Krauss [17]. The thickness of the gel was 1 mm. The amount of LPS per track was 5 μg.

RESULTS AND DISCUSSION

Rhodobacter are asporogenic microorganisms with rod-shaped or oval cells, 0.5–1.2 μm in diameter. They multiply by binary division; capsules and mucus can be formed; chains of cells can be present [18].

The presence of pigments is the distinctive feature of photosynthetic bacteria; therefore, the absorption spectra of cell suspensions are unique for each culture of cells [19]. The absorption spectra of strains B10 and PG in the wavelength range from 300 to 900 nm exhibited differences in the ratio of carotenoids and bacteriochlorophyll.

Many strains of *Rb. capsulatus* are known: B10, St. Louis, Sp11, 37b4, RC1, and others; they differ in the cell wall structure [1]. No chemotype-based classification of photosynthetic bacteria exists.

The study of the cultural characteristics of the strains under investigation revealed that on agarized medium strain B10 formed circular brick-colored colonies of the R type, firmly attached to the agar surface, with a wrinkled, semidry surface and uneven meandering edge. Strain PG form bright, smooth, circular, brick-colored colonies of the S type with an even edge, a uniformly elevated central section, and a brighter, flattened peripheral veil. The colonies are easily removed from the agar surface.

The sedimentation rate of the cells determined according to Lindberg makes it possible to determine the S or R chemotype of bacteria relatively accurately. For strain B10 cells, the sedimentation rate was 0.05 ± 0.012 mm/min, which makes it possible to place them among the R chemotype. For strain PG, this value was equal to 0.01 ± 0.006 mm/min, which indicates the RS chemotype of these cells.

For the study of the charged groups of the cell surface, EPM measurement was performed for the cells of strains B10 and PG depending on the pH of the suspension. The data presented in Fig. 1 show that the EPM of strain B10 cells had insignificantly higher negative values ($P < 0.01$) at neutral pH. Significant differences between the strains in the average EPM values of the cell populations emerged below pH 4.6 ($P < 0.001$). Strain B10 cells had a higher negative charge under these conditions. Upon further decrease in the pH of the cell suspensions, only strain PG cells, having passed the isoelectric state, acquired positive EPM; this find-

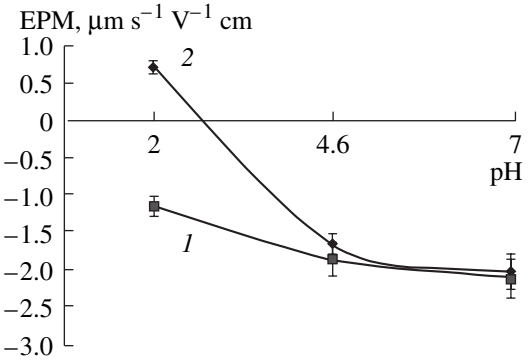

Fig. 1. EPM of the cells of strain B10 (*1*) and strain PG (*2*) depending on pH of the phosphate–citrate buffer with ionic force of 0.02.

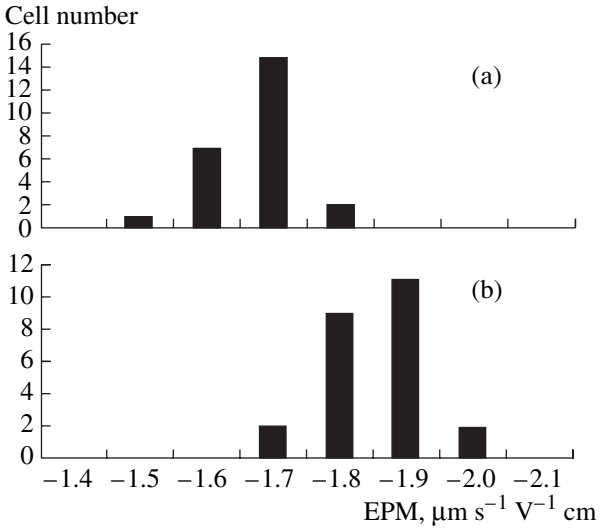

Fig. 2. Typical histograms of EPM distribution of the cells of strain PG (a) and strain B10 (b) in phosphate–citrate buffer, pH 4.6.

ing may indicate the presence of basic groups on their surface. In the cells of strain B10, pH decrease caused only a decrease in the EPM negative value. The graph of the pH dependence of strain B10 cells EPM demonstrates the presence of only negatively charged acidic groups (carboxyl, phosphate) on their surface. The shape of the curve EPM for strain PG is typical of a variety of bacterial cells, whose surface contains both negatively charged groups (carboxyl, phosphate) and positively charged ones (amino).

The typical histograms of the distribution of the EPM values of the cells of two *Rb. capsulatus* strains obtained at pH 4.6, where the most reliable differences in this parameter were observed, are presented in Fig. 2. It can be seen that the cell populations of the cultures under investigation exhibited a normal single-module distribution. The dispersion of the EPM values for both strains is possibly related to the presence of cells at different growth phases in both populations. EPM of *E. coli* cells increases in the course of culture growth due to the changing LPS content in the outer layer of the external membrane [20]. The histogram of distribution of EPM for strain B10 is shifted into the region of more negative values in comparison with that for strain PG; this result is caused by the presence of the LPS with a shortened structure in the composition of their cell walls [2]. The presence of both negatively and positively charged groups on the cell surface of strain PG in contrast to strain B10, which contains only negatively charged groups, may be another explanation of these results.

Batch cultures of *Rb. capsulatus* were used in our experiments; i.e., the cell population was grown under optimal conditions in a limited living space. The cells were collected in the exponential growth phase, which is characterized by a constant maximum division rate. We have demonstrated that the liberation of LPS into the medium was proportional to cell growth and that the maximum speed of LPS liberation corresponded to the exponential phase of growth (Fig. 3).

The electrophoretic properties of bacteria depend on the structure of LPS in their cell walls. We have therefore investigated the LPS which were liberated from the cell walls during bacterial growth and division. The analysis of the LPS which were liberated from the cells of strains B10 and PG into the medium during growth was performed using the reaction with the carbocyanine dye; it revealed differences in the position of the absorption maximum of the LPS–carbocyanine complexes (Fig. 4). For the LPS from strain B10, its wavelength was 458 nm, while for the LPS from strain PG, 468 nm. This shift of the absorption maximum in the spectrum of LPS–carbocyanine complexes towards the short-wave region indicates difference in the structures of the LPS of two strains, namely the more truncated structure of the LPS from strain B10 [21].

As was noted above, KDO is an inherent and required part of any LPS molecule. The molar fraction

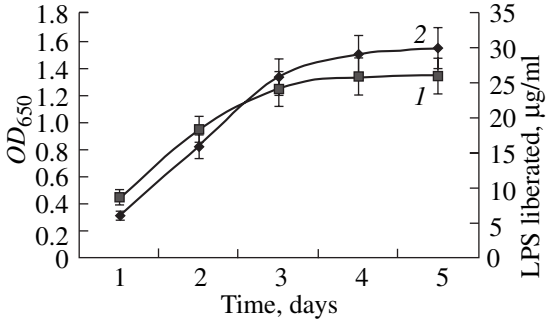

Fig. 3. Quantity of the liberated LPS (*1*) and the culture growth of strain PG (*2*). The average values of three independent experiments are presented.

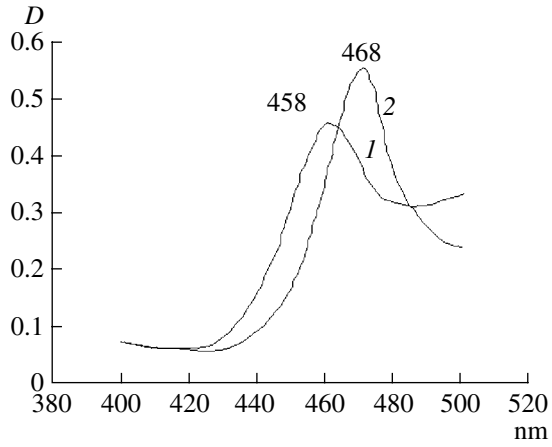

Fig. 4. Absorption spectra of the carbocyanine–LPS complexes from strains B10 (*1*) and PG (*2*).

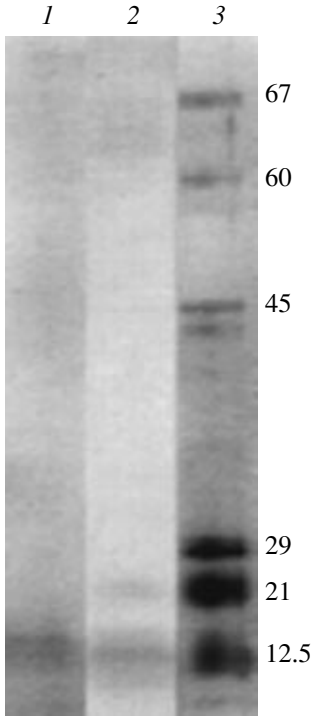

Fig. 5. Electrophoregram of the LPS isolated from: strain B10 (*1*), strain PG (*2*), and marker proteins (*3*). The molecular weights of marker proteins in kDa are given in the right column.

of KDO in the composition of the LPS molecules of the studied strains was determined by the spectroscopic method of KDO determination [16]. The results are presented in the table. It can be seen that the weight fraction of KDO in the LPS from strain PG was at least five times less than in the LPS from strain B10; this result indicates the presence of a polysaccharide fragment in the structure of the LPS from strain PG and, therefore, demonstrates that these bacterial strains belong to different chemotypes.

A more detailed analysis of the LPS from photosynthetic bacteria of different chemotypes, as for enterobacteria, is presently difficult. This is due to the fact that, unlike enterobacteria, the existence of different R-core structures is not confirmed for the majority of purple bacteria. Thus, the designations "Ra-, Rc-, Rd_2-, Re-like" are used for the LPS from photosynthetic bacteria solely as operational terms. For a number of photosynthetic bacteria, comparative analysis of the core structure and the representatives of *Enterobacteriaceae* with characterized chemotypes was performed; a good correlation was revealed between their chemotype, serotype, and LPS electrophoretic motility in gels [17].

LPS of the cells of the studied *Rb. capsulatus* strains were described by us using the method of electrophoresis in PAAG. It is evident from the electrophoregram (Fig. 5) that LPS from strain B10 contained only zones in the low molecular weight region, which correspond to lipid A. The same zones were revealed in the electrophoregram of strain PG LPS. The presence of additional bands in the region of 21 kDa indicated the presence of a polysaccharide fragment in the LPS from strain PG. These results make it possible to group the LPS from strain PG with the SR type, and those from strain B10, to R-LPS; they are in agreement with the data obtained by cell agglutination.

Thus, electrophysical and biochemical methods revealed that the strains of *Rb. capsulatus* under investigation differ in the structure of their cell walls and of the cell wall LPS. Strain B10 was grouped with the R, and strain PG, with the RS chemotype. The relation between the chemotype of photosynthetic bacteria of the genus *Rhodobacter* and the electrophoretic properties of the cells was established.

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KDO content in 1 mg of the LPS from different *Rb. capsulatus* strains

Rb. capsulatus LPS	A_{548}	KDO content, μmol
B10	0.55	0.0289
PG	0.105	0.0055

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