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EXPERIMENTAL ARTICLES

Characterization of *Pantoea agglomerans* Lipopolysaccharides

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Abstract—Lipopolysaccharides (LPS) from seven *Pantoea agglomerans* strains isolated from various plants were purified and chemically identified. LPS of the studied *P. agglomerans* strains were heterogeneous in monosaccharide composition. Thus, the LPS of *P. agglomerans* 8606 differed considerably from the LPSs of other strains, containing mannose as the predominant monosaccharide (69.8%), as well as ribose (15.1%) and xylose (12.6%), while the content of rhamnose, one of the predominant monosaccharides in other LPS samples, was 2.5%. Analysis of the fatty acid composition revealed the presence of C_{12}–C_{16} acids. In lipids A of all the studied strains, 3-OH-$C_{14:0}$ was the predominant acid (31.7 to 39.1%, depending on the strain). $C_{12:0}$ (8.2 to 31.5%), $C_{14:0}$ (12.9 to 30.8%), and $C_{16:0}$ acids (3.4 to 16.9%) were also revealed. The studied *P. agglomerans* strains fell into three groups according to their fatty acid composition. The differences stemmed from the presence or absence of two fatty acids, 2-OH-$C_{14:0}$ and $C_{16:1}$. Ouchterlony double immunodiffusion in agar revealed that all the LPS under study exhibited antigenic activity in homologous systems. The results of serological cross reactions indicated immunochemical heterogeneity of the species *P. agglomerans*. Comparative investigation of the complex of parameters of peripheral blood cells from a healthy donor before and after treatment with LPS solutions showed that the values of no parameters exceeded the normal range.

Keywords: Pantoea agglomerans, lipopolysaccharide, monosaccharide composition, fatty acid composition, serological activity, biological activity

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Representatives of *Pantoea agglomerans* are widespread in nature as commensals, epiphytes, or endophytes associated with many plants, warm-blooded animals, and insects. They were transformed into pathogens causing rots, leaf blotch, and galls (swellings) in plants and opportunistic infections in humans and animals. The transformation of bacteria into host-specific, gall-forming pathogens occurred due to the evolution of the unique plasmid of pathogenicity, pPATH [1]. Lipopolysaccharides (LPS) are known to play a key role in the host–pathogen interactions of both animals and plants [2, 3]. Specific features of the LPS structure can be of chemotaxonomic importance, in particular, for representatives of *P. agglomerans*, which have long been assigned to various bacterial genera and species: *Enterobacter agglomerans*, *Erwinia herbicola*, and *Erwinia milletiae*. In 1989, a new genus *Pantoea* within the family *Enterobacteriaceae* was described, which contained the rather heterogeneous species *P. agglomerans* [4].

Until now, there was only scarce information on the isolation and characterization of LPS from phytopathogenic representatives of *P. agglomerans*; the goal of the present work was therefore to obtain LPS from *P. agglomerans* strains isolated from different plants, to carry out their chemical identification, and to establish serological interrelations between them.

MATERIALS AND METHODS

Research subjects were seven strains of *P. agglomerans* obtained from the Collection of the Department of Phytopathogenic Bacteria, Institute of Microbiology and Virology, NAS of Ukraine, which were isolated from tomato (Uman', Ukraine) (7960), apple tree (Minsk, Belarus) (7969), oat seeds (Romania; kindly provided by Dr. T. Lazer) (8456, 8488, and 8490), cotton plant (St. Petersburg, Russia; kindly provided by K.L. Khetagurova) (8606), and strain 8674 (LMQ 2565 = NCCPPB 2971, type strain) (Belgium, kindly provided by Dr. J. De Ley).

Bacteria were grown on potato agar at 28–30°C for 36 h. The cells were harvested by centrifugation (5000 *g*, 20 min) and dried with acetone and diethyl ether.

Isolation of LPS. LPS was extracted from dried cells with 45% aqueous solution of phenol at 65–68°C; aqueous fractions were dialyzed sequentially against tap and distilled water for phenol removal [5].

Assays of carbohydrates, nucleic acids, and protein. The amount of carbohydrates was determined by the Dubois method [5]. The results were assessed spectrophotometrically (490 nm) by color change resulting

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from the reaction of phenol with sulfuric acid. The content of carbohydrates was determined in accordance with the standard calibration curves plotted for glucose.

The content of nucleic acids and proteins was analyzed by the methods of Spirin [5] and Lowry with the Folin reagent [5], respectively.

Identification of neutral monosaccharides was carried out after hydrolysis of the preparations in 2 N HC1 (5 h, 100°C). Monosaccharides were analyzed as polyol acetates [6] on an Agilent 6890N/5973 inert chromatography–mass spectrometry system equipped with a DB-225mS column (30 m × 0.25 mm × 0.25 μm); the carrier gas was helium at a flow rate of 1 mL/min. The temperatures of the evaporator, interface, and thermostat were 250, 280, and 220°C, respectively (isothermal mode). The sample was injected at a flow distribution of 1 : 100. Monosaccharides were identified by comparing the retention times of polyol acetates in the experimental and standard samples and using the ChemStation database. The quantitative ratios of individual monosaccharides were expressed as a percentage of the sum of peak areas.

Determination of the fatty acid composition was performed after hydrolysis of the LPS preparation in a 1.5% solution of acetyl chloride in methanol (100°C, 4 h); the fatty acid methyl esters were analyzed on an Agilent 6890N/5973 inert chromatography–mass spectrometry system with a HP-5MS column (30 m × 0.25 mm × 0.25 μm), at the temperature mode of 150–250°C and temperature gradient, 4°C; the carrier gas was helium at flow rate of 1.2 mL/min. The evaporator temperature was 250°C; flow distribution was 1 : 100. Fatty acids were identified using the PC database and the standard mixture of the fatty acid methyl esters. The quantitative ratios of individual fatty acids were expressed as a percentage of the sum of peak areas.

Immunological studies. The O-antiserum was obtained to heated *P. agglomerans* cells (2.5 h, boiling water bath). The rabbits were immunized intravenously five times with 4-day intervals; cell concentration was 2×10^9 cells/mL (from 0.1 to 1.0 mL).

The antigenic activity of LPS was studied by the method of double immunodiffusion in agar according to Ouchterlony [7].

The analysis of phagocytic activity of neutrophiles is based on the ability of phagocytes to ingest the latex particles which are stained blue by the Romanovsky–Giemsa reagent [8, 9]. The results were determined microscopically for 100 neutrophiles. The phagocytic index (P_i), which characterizes the number of cells capable of active ingestion of particles, and the phagocytic number (P_n), the number of latex particles which were ingested by one phagocyte, were estimated. Both parameters characterized the ingestion capability of the blood phagocytic cells (neutrophiles and monocytes). The phagocytic number is normally from 1.5 to 8.0 U, and the phagocytic index is 40–80%.

To evaluate the parameters of innate immunity, we used the nitro blue tetrazolium (NBT) test, which characterized the oxygen-dependent metabolism of neutrophiles [9]. We used the NBT-spontaneous test; when NBT contacted with the activated neutrophiles, it was reduced to diformazan, which was deposited as granules insoluble in water and most organic solvents inside or at the surface of the cells. The intensity of reaction was characterized by the amount of diformazan formed. The share of diformazan-containing cells of the total number of the blood phagocytic cells was determined microscopically and expressed as percentage of the total number of blood phagocyte cells. To evaluate the activity of peroxidase system, the mean cytochemical coefficient (MCC) was calculated:

$$\mathrm{MCC} = \frac{1\alpha_{1/4} + 2\alpha_{2/4} + 3\alpha_{3/4} + 4\alpha_{4/4}}{10_0},$$

Where $\alpha_{1/4}$, $\alpha_{2/4}$, $\alpha_{3/4}$, and $\alpha_{4/4}$ are the numbers of cells in which diformasan granules occupy 1/4, 2/4, 3/4, and 4/4 of the cell area, respectively.

MCC value varies normally from 0.06 to 0.7; the number of NBT-positive cells is 10–30%.

The effect of LPS on different lymphocyte populations was assessed by the level of expression of CD_3- and CD_{22}-receptors on T- and B-lymphocytes with the use of the reaction of rosette formation with the monoclonal antibodies to the above receptors [10]. CD-diagnosticum for the antibodies was introduced into round-bottom wells of an immunological plate (or microtubes) by 0.25 mL, supplemented by an equal volume of lymphocyte mixture, incubated for 25 min at 37°C, centrifuged at 500–1000 *g* for 3 min, and placed into a refrigerator for 1 h at 4°C. The lymphocyte precipitates treated with the monoclonal antibodies were dried, fixed with alcohol, and stained by Romanowsky–Giemsa so that the nuclei of the lymphocytes were clearly visible.

Percentage of T-lymphocytes corresponded to the percentage of lymphocytes forming rosettes with CD_3-diagnosticum; its normal range for adults is from 50 to 80%. Percentage of B-lymphocytes corresponded to the percentage of lymphocytes forming rosettes with CD_{22}-diagnosticum; its normal range for adults is from 17 to 31%.

RESULTS AND DISCUSSION

It was found that LPS yield in *P. agglomerans* varied from 5.2 to 14.0%; it exceeded the average values typical of the other members of *Enterobacteriaceae* (5%), but was lower than that in some *Pseudomonas* strains (up to 32%).

The LPS preparations were characterized by a rather high content of nucleic acids (up to 30%), which might be due to the LPS extraction procedure

Table 1. Chemical characteristics of *P. agglomerans* LPS

Parameters (% of dry LPS)	Strains						
	7960a	7969	8456	8488	8490	8606	8674t
LPS yield	5.9	9.5	14.0	10.9	5.2	8.4	6.8
Carbohydrates	32.0	29.0	24.0	38.0	42.0	35.0	42.0
Protein	–		Tr	–	Tr	0.8	–
Nucleic acids	1.6	2.9	3.1	3.4	13.5	8.1	7.7
KDO	2.2	0.5	1.6	0.8	0.7	1.3	0.4

"Tr" stands for "trace amount"; "–" indicates the absence of a component.

Table 2. Monosaccharide composition of *P. agglomerans* LPS

Monosaccharides (% of the sum of peak areas)	Strains *P. agglomerans*						
	7960a	7969	8456	8490	8606	8488	8674
Rha	15.4	18.7	4.9	12.3	2.5	21.9	38.6
Fuc	14.8	14.9	–	14.5	–	25.9	–
Rib	1.7	1.1	22.3	29.8	15.1	2.8	6.2
Xyl	–	–	–	–	12.6	–	–
Man	23.5	24.5	–	21.2	69.8	30.9	–
Gal	4.5	3.2	11.5	0.8	–	2.9	5.9
Glñ	16.5	16.8	23.8	11.8	–	12.8	40.5
Hep	23.6	20.8	37.5	9.6	–	2.8	8.8

"–" Indicates the absence of a component.

applied. After purification of the preparation by ultracentrifugation (three times at 104000 *g*, 4 h), LPS contained a significant amount of carbohydrates (29.0–42.0%), traces of protein (up to 0.8%), and nucleic acids; the content of the latter in some strains remained rather high (7.7–13.5%) (Table 1).

The LPSs of the studied strains were rather heterogeneous in monosaccharide composition (Table 2). The LPS of strain 8606 differed considerably from those of the other strains by predominance of mannose (69.8%); it contained ribose (15.1%) and xylose (12.6%), whereas the amount of rhamnose, which prevailed in the other LPSs studied, was as low as 2.5%. Fucose (from 14.5 to 25.9%) was revealed in LPSs of four strains (7960a, 7969, 8490, and 8488). Mannose (from 21.2 to 69.8%) was found in LPSs from all studied *P. agglomerans* strains, except for strains 8456 and 8674. The heptose content of LPSs depended on the strain and varied from 2.8 to 37.5% (Table 2). The amount of 2-keto-3-deoxyoctulosonic acid (KDO), a typical LPS component of gram-negative bacteria, ranged in the studied strains from 0.4 to 2.2% (Table 1).

Thus, the isolated LPSs contained all the monosaccharide components typical of this group of glycopolymers.

The LPSs were shown to include fatty acids with chain lengths from 12 to 16 carbon atoms; lipid A in all the studied strains contained 3-OH-$C_{14:0}$ acid as the predominant component (from 31.7 to 39.1% depending on the strain) as well as $C_{12:0}$ (from 8.2 to 31.5%), $C_{14:0}$ (from 12.9 to 30.8%), and $C_{16:0}$ (from 3.4 to 16.9%) (Table 3). Based on fatty acid composition, all the studied *P. agglomerans* strains may be divided into three groups, depending on the presence or absence of two fatty acids: 2-OH-$C_{14:0}$ and $C_{16:1}$. The first group included the strains (8674, 8606, 8456, and 7960a), in which lipid A contained $C_{16:1}$, but not 2-OH-$C_{14:0}$ acid; the second group included strains 8488 and 7969, which contained 2-OH-$C_{14:0}$ (3.8 and 2.5%, respectively), but not $C_{16:1}$ acid; the third group included only strain 8490 in which lipid A contained neither 2-OH-$C_{14:0}$ nor $C_{16:1}$ acids.

Traits of the LPS composition determined serological specificity of microbial cells due to the presence of

Table 3. Fatty acid composition of lipid A of the LPS from *P. agglomerans*

LPSs from strains:	Fatty acids (% of the sum of peak areas)					
	$C_{12:0}$	$C_{14:0}$	2-OH-$C_{14:0}$	3-OH-$C_{14:0}$	$C_{16:1}$	$C_{16:0}$
8674	8.2	28.3	–	32.7	27.4	3.4
8606	25.9	29.6	–	36.2	4.2	4.1
8456	25.6	28.9	–	35.9	4.8	4.8
7960a	17.6	30.8	–	38.9	9.3	3.4
8490	30.4	23.1	–	31.7	–	14.8
8488	31.5	12.9	3.8	34.9	–	16.9
7969	27.4	17.7	2.5	39.1	–	13.1

"–" Indicates the absence of a component.

antigenic determinants, which can be recognized by the cells of other organisms. Immunochemical properties of LPSs were studied using the polyclonal O-antisera obtained from the rabbits immunized with heat-treated cell suspensions of *P. agglomerans* strains; LPSs isolated from the studied strains served as antigens. The O-antiserum titers determined by the ring precipitation reaction varied from 2.5×10^{-5} to 5.0×10^{-5}, depending on the strain. Reaction of double immunodiffusion in agar by Ouchterlony showed that all the studied LPSs exhibited antigenic activity in homologous systems (Fig. 1). It is known that a method of serological cross reactions can be used in taxonomy of bacteria. Based on the obtained results (Fig. 1), strains of *P. agglomerans* may be divided into five serogroups. The antiserum to the type strain *P. agglomerans* 8674 interacted not only with the homologous strain, but also with LPS from strain 7960a, which indicated the presence of common antigenic determinants in these strains (the first serogroup). The second serogroup included two strains (8606 and 8674); the antiserum to *P. agglomerans* 8606 also exhibited a cross reaction with the LPS from the type strain 8674. Strains *P. agglomerans* 7960a, 8606, and 8674 formed the third serogroup: antiserum to *P. agglomerans* 7960a contained common antigenic determinants with the LPSs from strains 8606 and 8674. The antiserum to *P. agglomerans* 8456 interacted also with the LPS from strain 7960a (the fourth serogroup). It should be noted that the LPS from the type strain *P. agglomerans* 8674 interacted also with antisera to the other two strains (7960a and 8606), and the LPS from strain *P. agglomerans* 7960a interacted also with antisera to strains 8674 and 8456. We assigned strains *P. agglomerans* 7969, 8488, and 8490 to the fifth serogroup; in this case, each of three antisera interacted with each of three LPSs studied.

The results obtained are indicative of immunochemical heterogeneity of the species *P. agglomerans*.

In the course of the infection processes, the reaction of cellular factors of innate immunity to the pathogen is of great importance for surmounting the defense functions of the organism immunoreactivity. The main elements of innate immunity responsible for the first line of defense against infection include phagocytes (macrophages, neutrophiles, natural killers, dendritic cells, etc.) [11, 12]. Protective functions of these cellular effectors are based on the phagocytic process, i.e., their ability to engulf and then digest alien material [12, 13].

Lipopolysaccharides are known to affect the immune system of microorganisms, its polyclonal activation, stimulate or suppress its response to certain antigens, induce polyclonal immune tolerance, show slight effect on T-lymphocytes, increase phagocytic activity of neutrophiles, activate the total coagulation potential of blood, increase microcirculatory disturbances, and exhibit antitumor activity.

We studied the effects of LPSs from two *P. agglomerans* strains (7960a and 8674) on such blood parameters as phagocytic activity of neutrophiles, oxygen-stimulating activity of phagocytic cells, and phenotyping of T- and B-lymphocytes. The blood samples taken from a healthy young man (22 years old) were treated with the lipopolysaccharide solutions in concentrations of 1 : 10 and 1 : 100.

The effect of LPSs on the peripheral blood phagocytes was studied by the spontaneous nitro blue tetrazolium (NBT) test (Fig. 2). This test evaluates the activation of oxygen-dependent mechanisms responsible for the killing of nonactivated phagocytes and characterizes the level of activation of intracellular bactericidal systems. It was found that the LPS from *P. agglomerans* 7960a (in both concentrations) slightly decreased the percentage of NBT-positive cells and

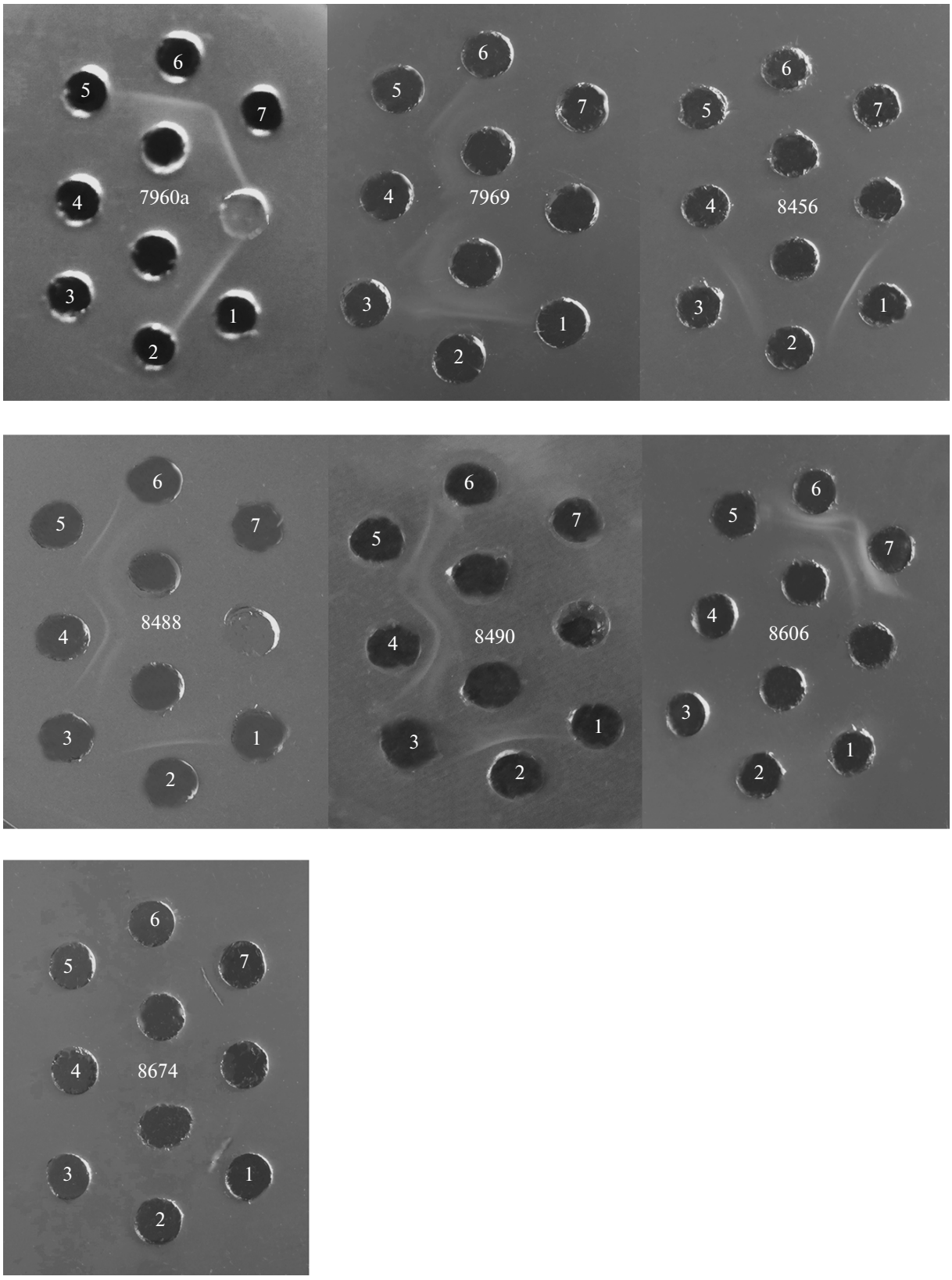

Fig. 1. Reaction of double immunodiffusion in agar by Ouchterlony between the antisera to *P. agglomerans* 7960a, 7969, 8456, 8488, 8490, 8606, and 8674 and the LPSs from *P. agglomerans* 7960a (1), 7969 (2), 8456 (3), 8488 (4), 8490 (5), 8606 (6), and 8674 (7).

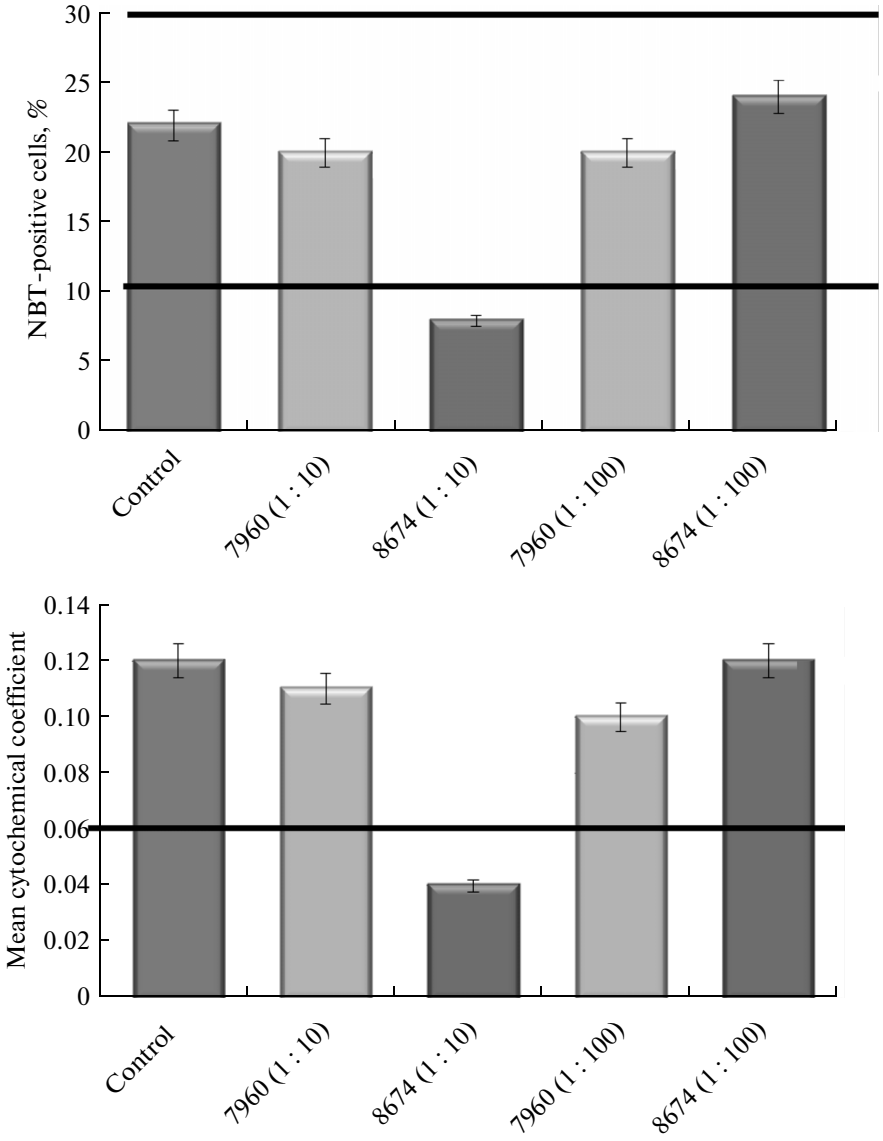

Fig. 2. The oxygen-dependent activity of the peripheral blood phagocytic cells determined by the reaction with nitro blue tetrazolium (NBT test).

the value of the mean cytochemical coefficient. The LPS from *P. agglomerans* 8674 in dilution of 1 : 10 decreased both parameters, whereas in concentration of 1 : 100 it increased the percentage of NBT-positive cells, but showed no effect on the MCC value. It should be noted that changes in all parameters caused by LPSs were within the normal range except for a decrease in the percentage of NBT-positive cells and the MCC value in the presence of LPS from *P. agglomerans* 8674 in concentration of 1 : 10. In a normal state, MCC coefficient varies from 0.06 to 0.7, and percentage of NBT-positive cells ranges from 10 to 30%.

It is known that the parameters of the NBT test are increased at the beginning of acute bacterial infections and pyoinflammatory processes and decreased in the case of subacute and chronic infection processes indicating the insufficiency of phagocytosis [14].

Evaluation of the immune state of an organism, which is usually disturbed under inflammatory infectious diseases, includes assessment of the phagocytic characteristics of immune system, such as the phagocytic index (P_I), which is expressed as the percentage of the phagocytosis-involved cells of the total cell number, and the phagocytic number (P_N), the mean number of intracellular bacteria, which is expressed as a result of division of the total number of captured bacterial cells by the number of cells involved in phagocytosis. A phagocytic number characterizes the ingesting capability of neutrophiles at the terminal phase of phagocytosis; a phagocytic index of neutrophiles characterizes the ability of phagocytes to digest ingested

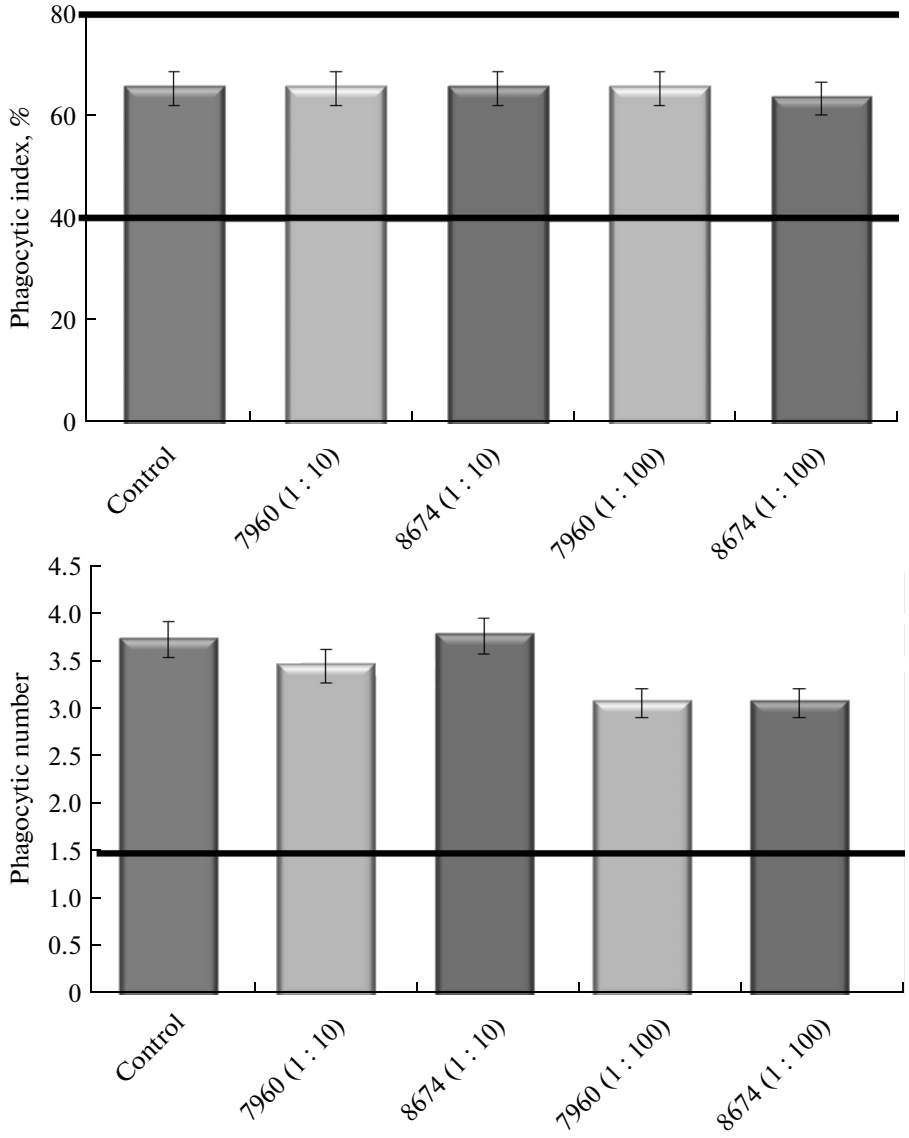

Fig. 3. Phagocytic activity of neutrophiles.

microorganisms at the terminal phase of phagocytosis. These parameters, together with the other phagocytic characteristics, assure valid evaluation of the phagocytic reactions.

It was found that LPSs from *P. agglomerans* 7960a (in both concentrations) and 8674 (in concentration of 1 : 10) showed no effect on the phagocytic index, whereas the LPS from *P. agglomerans* 8674 in concentration of 1 : 100 decreased P_I value by 2% as compared with the control (Fig. 3).

The difference between P_N values in the control and in the presence of LPSs from *P. agglomerans* 7960a and 8674 (in concentrations of 1 : 100) was 0.67 units; this difference decreased to 0.28 and 0.04 units in the presence of LPSs from *P. agglomerans* 7960a (in concentration of 1 : 10) and 8674 (in concentration of 1 : 10), respectively.

It was found that all parameters of the phagocytic activity of neutrophiles in the presence of the studied LPSs were within the normal range (from 1.5 to 8.0 units for P_N and from 40 to 80% for P_I).

Bacterial LPSs are known as stimulators of innate and acquired immunity in various eukaryotes [15]. T-lymphocytes play a key role in the regulation of practically all elements of immunity; in particular, they synthesize pro- and anti-inflammatory cytokines and regulate the synthesis of other cells. The ratio of these cytokines determines the direction of metabolic processes in the cells, their resistance to the action of apoptogenic factors, and many other physiological functions of the organism, which have a direct effect on the reparative processes in the tissues. B-cells are a

Table 4. Effect of LPS treatment on characteristics of the peripheral blood cells

Parameters	Control	7960a		8674		Normal range
		1 : 10	1 : 100	1 : 10	1 : 100	
NBT-positive cells, %	22	20	20	8	24	10–30
Mean cytochemical coefficient, units	0.12	0.11	0.1	0.04	0.12	0.06–0.7
Phagocytic index, %	66	66	66	66	64	40–80
Phagocytic number, units	3.76	3.48	3.09	3.80	3.09	1.5–8
T-lymphocytes, %	56	42	35	50	28	50–80
B-lymphocytes, %	24	25	20	30	10	17–31

functional type of lymphocytes playing an important role in providing humoral specific immunity.

The characteristic feature of B- and T-lymphocytes is the presence of a number of antigenic markers, in particular, CD_{22}- and CD_3- receptors, respectively. It is known that the CD_3 protein complex associated with an antigen-specific T-cell receptor is a major functional marker of T-lymphocytes. It promotes signal transduction from the cell membrane to the cytoplasm. The B-lymphocyte receptor CD_{22} is a carbohydrate-binding transmembrane protein from the lectin family. It is a regulatory molecule, which prevents overactivation of the immune system and development of autoimmune processes.

Phenotyping of B- and T-lymphocytes was carried out with the use of monoclonal antibodies to the C_{22}- and CD_3-receptors by employing the direct rosette formation test based on the differences in the receptor structure of leukocytes (lymphocytes, granulocytes, and monocytes), which are capable of spontaneous attachment of erythrocytes to their surface. In this case, rosette-like figures are formed, in which the leukocyte located in the central part is surrounded by at least three to five erythrocytes. According to this method, a suspension of leukocytes (lymphocytes) obtained from a patient is incubated with a suspension of sheep erythrocytes, which were preliminarily either treated or untreated with the antigen, and then the number of rosette-forming cells is calculated.

In the presence of LPSs from both strains (7960 and 8674) in both concentrations, the percentage of T-lymphocytes was lower than that in control (Fig. 4). Except for LPS from strain 8674 (in concentration of 1 : 10), all the studied LPSs decreased the number of T-lymphocytes below that in the normal state (50%). The number of B-lymphocytes in the presence of the studied LPSs (except for LPS from strain 8674 in concentration of 1 : 100) were within the normal range, which varies from 17 to 31%.

Comparative data on the effects of LPSs on characteristics of the peripheral blood cells taken from a healthy donor indicated that almost all parameters were within the normal range (Table 4). Only in one case, when reaction of the lymphocyte phenotyping by the rosette formation test with monoclonal antibodies was performed in the presence of the LPS from *P. agglomerans* 7960a (in concentrations of 1 : 10 and 1 : 100), was the percentage of T-lymphocytes lower than that in the normal state (50–80%). The NBT test revealed that the LPS from *P. agglomerans* 8674 in concentration of 1 : 10 suppressed oxygen-dependent activity of the peripheral blood phagocytes. This LPS preparation in concentration of 1 : 100 decreased amounts of T- and B-lymphocytes below the normal range. It is known that a decrease in the B-lymphocyte number is typical of acute and chronic viral infections, spleen removal, the immune system neoplasms, and in the case of treatment with cytostatic drugs. A decrease in the absolute number of T-lymphocytes can be indicative of insufficiency of the cell immunity.

We have earlier revealed that the LPS from *Escherichia coli* M-17 in dilutions of 1 : 10 and 1 : 100, similar to the LPS from *P. agglomerans* 7960a, decreased expression of CD_3-receptors on T-lymphocytes [16]. The effects of various concentrations of LPS from *E. coli* M-17 on expression of C_{22}-receptors on B-lymphocytes were different: the expression was increased twofold at LPS concentration of 1 : 10 and

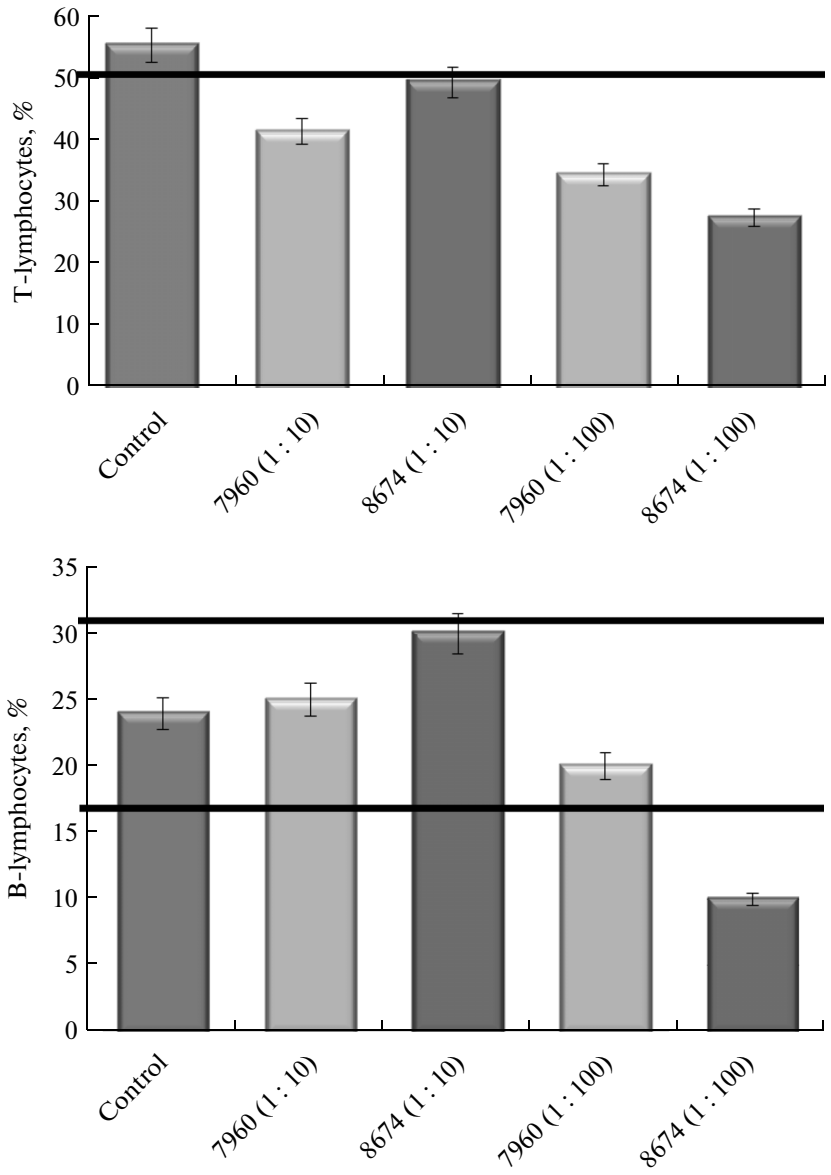

Fig. 4. Phenotyping of lymphocytes by the rosette formation test with the particles covered with monoclonal antibodies.

suppressed by more than 80% at the preparation concentration of 1 : 100. Thus, it can be concluded that the effect of LPSs on expression of CD_3- and CD_{22}-receptors on T- and B-lymphocytes depended on the preparation concentration.

According to the literature data [17], microbial lipopolysaccharides penetrate into blood and stimulate production of inflammatory cytokines in the LPS-sensitive cells, which induce the acute-phase liver response, including lipoprotein synthesis. Lipoproteins promote the formation of the LPS complexes with the very low-density lipoproteins and chylomierons, which penetrate into blood and neutralize the LPS, decreasing its toxicity. These complexes are detoxified in the liver, reducing its response to subsequent stimulatory action of cytokines. Based on the obtained data, it can be assumed that LPSs from *P. agglomerans* in the given concentrations would have no severe impact on a human organism.

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