https://doi.org/10.15407/microbiolj86.06.020

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CHARACTERISTICS OF *ESCHERICHIA COLI* K LIPOPOLYSACHARIDE

Previously, the authors isolated from the intestine of the chestnut moth caterpillars two cultures of bacteria — Pseudomonas putida and Escherichia coli. Since in interactions with plants or other living beings, lipopolysaccharides are involved, earlier we have characterized the lipopolysaccharide of one of the strains — Pseudomonas putida. The ***aim*** *of this study was to characterize the lipopolysaccharide of a new strain of Escherichia coli K, in particular its composition and biological activity.* ***Methods.*** *Lipopolysaccharide (LPS) was obtained from cells by water-phenol extraction, heterogeneity was determined by SDS-PAAG electrophoresis, monosaccharide and fatty acid compositions were determined by chromatography-mass spectrometry, and serological activity — by immunodiffusion in agar. Determination of the osmotic resistance of erythrocytes was carried out according to the research by Bazarnova et al.* ***Results.*** *In purified LPS, 29.46% carbohydrates, 4.9% nucleic acids, and 9.53% proteins were found. The content of 2-keto-3-deoxyoctonic acid (KDO) and heptose, characteristic of LPS components, was 0.03% and 5.64%, respectively. Identification of the monosaccharide composition indicates that galactose dominates in LPS — 70.87%. Fucose, ribose, and glucose were found in smaller quantities (11.86, 10.11, and 7.16%, respectively). The hydroxy acids 2-OH-$C_{12:0}$ — 32.23%, 2-OH-$C_{14:0}$ — 25.07% and 3-OH-$C_{14:0}$ — 7.26% were identified in the composition of the LPS preparation under study. $C_{14:0}$ — 10.69%, $C_{17:0}$ — 5.35%, $C_{16:0}$ — 3.15%, $C_{15:0}$ — 3.04%, and unidentified fatty acid — 13.21% were also detected. SDS-PAGE electrophoresis showed that the studied E. coli LPS is represented by a heterogeneous population, which includes two main types of molecules: high-molecular-weight S-LPS with O-chains of various lengths and low- molecular-weight R-LPS, which does not contain O-specific polysaccharide chains. The tested preparation of E. coli LPS turned out to be*

Citation: Varbanets L.D., Brovarskaya O.S., Garkava K.G., Tymoshenko U.V. Characteristics of *Escherichia coli* K Lipopolysacharide. *Microbiological journal.* 2024 (6). P. 20—29. https://doi.org/10.15407/microbiolj86.06.020

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pyrogen-free: it did not reach either the level of pyrogenicity or the level of «Pyrogenal» (pharmaceutical drug), the active component of which is Shigella typhi LPS. The determination of osmotic resistance of erythrocytes showed that a high percentage of hemolysis is observed in the erythrocytes of all donors after treatment with solutions of LPS preparations, both native and modified. ***Conclusions.*** *The obtained data indicate a high biological potential of the influence of lipopolysaccharides on the resistance of erythrocytes, which gives reason to attribute this criterion to possible prognostic factors of the risk of osmotic lysis.*

Keywords: *Escherichia coli K, lipopolysaccharide, monosaccharide, fatty acid composition, pyrogeneity, heterogeneity, osmotic resistance of erythrocytes.*

The study of enterobacteria is almost 200 years old, but even today they remain in the center of attention of researchers. This is due to the fact that the role of enterobacteria in human pathology has significantly increased over the past 20—30 years. In various conditions accompanied by weakening resistance of the macroorganism, enterobacteria are able to penetrate tissues and tissue liquids, can make up to 80% of clinical isolates of all gram-negative bacteria and cause 50% of all cases of bacteremia, up to 70% of gastroenteritis and more than 70% of urinary tract infections. Possessing a wide range of environmental tolerance, representatives of enterobacteria are able to occupy other ecological niches, in particular soil, air, insects, plants, animals, and new habitats of enterobacteria in the natural environment have also been discovered. Thus, no less than 18 species of representatives of 10 genera of enterobacteria were found in the body of honey bees. Such a wide distribution of enterobacteria indicates the expediency of studying them both at the phenotypic and biochemical levels. Previously, the authors, studying chestnut leaves, isolated from the intestine of the chestnut moth caterpillars two cultures of bacteria: *Pseudomonas putida* and *Escherichia coli*. Since lipopolysaccharides (LPS) are involved in interactions with plants or other living beings, we have characterized (Brovarska et al., 2020) of one of the strains — *Pseudomonas putida*. The aim of this study was to characterize LPS of a new strain of *Escherichia coli*, in particular its composition and biological activity.

Materials and Methods. The object of the study was a culture of *Escherichia coli* K isolated from chestnut leaves, into which it entered with the feces of the caterpillars of the chestnut transient moth (*Cameraria ohridella Deschka & Dimic*) (Fig. 1).

The culture was grown on meat peptone agar (MPA) at room temperature for 48 h on mattresses. LPS was obtained by water-phenol extraction of cells dried with acetone and ether according to the Westphal method (Westphal, 1965).

Determination of the chemical composition of LPS was carried out by conventional methods: total carbohydrates — according to Dubois (Dubois, 1956), proteins — according to Lowry (Lowry, 1951), nucleic acids — according to Spirin (Spirin, 1958). The presence of 2-keto-3-deoxyoctonic acid (KDO) in LPS was determined by the reaction with thiobarbituric acid (Varbanets et al, 2006) and heptoses — according to Dische (Dische, 1949).

To obtain succinylated LPS, 2 mL of distilled pyridine was added to native LPS (20 mg) and kept at 100 °C for 1 min. After cooling to room temperature, 1.6 mL of a solution of succinic anhydride in pyridine was added to the sample (prepared before the experiment, in the ratio of 100 mg and 1.6 mL, respectively), held for 30 s at 100 °C in a water bath and hydrolyzed for 3 h at 56 °C. After cooling, the mixture was neutralized with 0.1 N NaOH solution to pH 7.0 and dialyzed against distilled water for 48 hours at room temperature. The final product was lyophilized.

The monosaccharide composition (Albersheim et al, 1967) was analyzed as polyol acetates on an Agilent 6890N/5973 inert chromatography—mass spectrometry system equipped

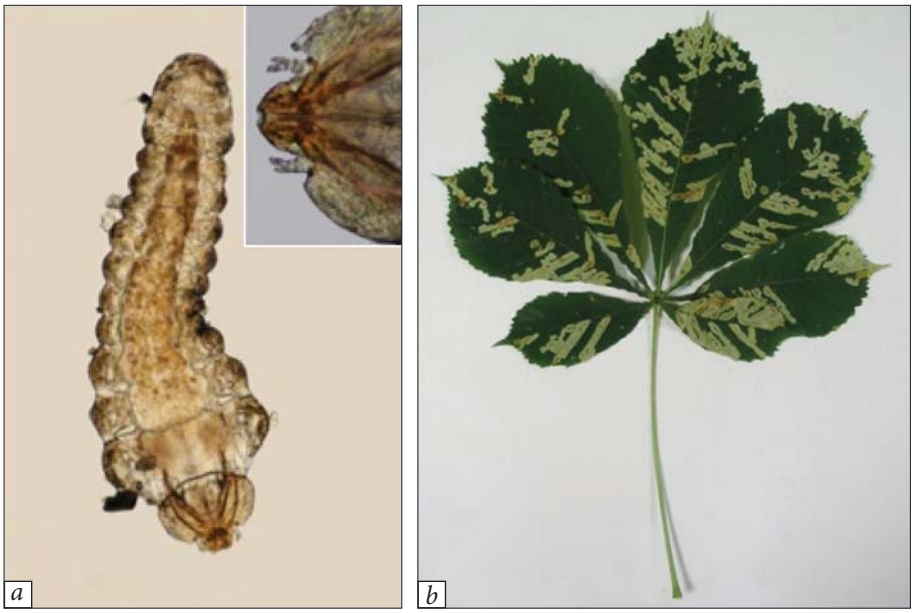

Fig. 1. The general appearance of the caterpillar and the structure of the oral apparatus at the early stages of development (*a*). The initial stage of development of the chestnut transient moth on a chestnut leaf (*b*) (Brovarska et al., 2020)

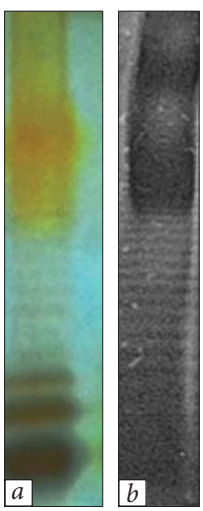

Fig. 2. Electrophoresis of *E. coli* LPS in a polyacrylamide gel in the presence of SDS: *a* — LPS *E. coli* K, *b* — LPS *E.coli* 126

with a DB 225 mS column (30 m × 0.25 mm × × 0.25 μm); the carrier gas was helium at a flow rate of 1 mL/min. The identification of monosaccharides was performed by comparison of the retention times with the authentic samples.

Fatty acid composition was determined by methanolysis of LPS with 1.5% acetyl chloride in methanol (100 °C, 4 h). The identification of fatty acids was performed as methyl esters by GLC-MS on Agilent 6890N/5973 inert chromatography-mass spectrometry system equipped with an HP 5ms column (30 m × 0.25 mm × 0.25 nm) using the temperature program from 150 to 250 °C at 4 °C/min; helium was used as a carrier gas at a flow rate of 1.2 mL/min. The evaporator temperature was 250 °C, and flow distribution was 1:100. For identification of fatty acids, the standard mixture of fatty acid methyl esters (Supelko, USA) and the available databases were used (Varbanets et al, 2006).

The quantitative content of individual monosaccharides and fatty acids was expressed as % to the total sum of peak areas.

Polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAAG electrophoresis) was performed according to Laemmli (Laemmli, 1970) (4% concentrating and 12% separating gel, current 30 mA). The

load on the gel lane was 20 μg. To visualize LPS, the gels were stained with silver salts according to Tsai's recommendations (Tsai et al, 1982) modified by Kulikov (Kulikov et al., 2019).

In the study of biological and functional activity, the following LPS concentrations were used: the minimal pyrogenic dose was 7.5×10^{-3} μg/mL per 1 kg of animal weight (pyrogenicity) and 1 mg/mL (serological studies). The work was conducted in accordance with the «General Ethical Principles of Animal Experiments».

The LPS pyrogenicity was determined in rabbits (breed Chinchilla) weighing 2.0—3.5 kg. The temperature was measured in 30 min intervals before the injection and in 1 hour intervals after the injection. The LPS solutions under test were considered apyrogenic if the temperature did not increase more than by 1.4 °C for 3 hours (Varbanets et al., 2006).

O-antiserum to a heated (2.5 h, 100 °C) culture of *E. coli* K grown on MPA was obtained by four intravenous injections of increasing doses of a suspension of microbial bodies (from 500 thousand to 2 billion cells/mL at 0.5 mL per rabbit) with an interval between the injections of 5 days. On the 5^{th} day after the last injection, a blood sample (20—30 mL) was taken from the vein of the ear to obtain O-antisera. The titer of sera was determined by the ring precipitation reaction. The antigenic activity of LPS was studied by the method of double immunodiffusion in agar according to Ouchterlony (Ouchterlony, 1962).

To study the effect of LPS on the osmotic resistance of erythrocytes, the blood of donors (8 people) was used. When planning the work, positive decisions of the medical ethics committee were received regarding compliance with the ethical standards in scientific research involving humans, which corresponds to the principles of the Declaration of Helsinki «Ethical principles of medical research involving humans as research objects», the Convention for the Protection of Human Rights and Dignity of the Human Being with regard to the Application of Biology and Medicine, otherwise known as the European Convention on Bioethics, and the relevant laws of Ukraine. Individuals (8 donors) who were examined within the framework of this work provided a written informed consent for participation in the scientific clinical research (trials).

To determine the osmotic resistance of erythrocytes (ORE), heparinized blood was used according to the research by Bazarnova et al. (Bazarnovoi, Morozova, 1988). Heparin is the optimal anticoagulant for determining the level of erythrocyte hemolysis. The osmotic resistance of erythrocytes was determined in blood samples immediately after collection and in samples incubated at a temperature of 37 °C. Since it is not always possible to detect functional changes in the cytoplasmic membrane of erythrocytes in a freshly collected blood sample, it is necessary to conduct an additional examination of the osmotic resistance of erythrocytes (ORE) after incubating the sample for 24 hours at 37 °C (Bazarnovoi et al., 1988). The reaction was carried out in NaCl dilutions from 0.1% to 0.85% in order to choose the NaCl dilution where the maximum resistance of erythrocytes would be recorded.

Normally, in freshly collected blood, the maximum ORE value is at the level of 0.35—0.40% NaCl solution. In blood samples incubated at 37 °C for 24 hours, the maximum ORE value is at the level of 0.40—0.45% NaCl solution. This is a classic technique for determining ORE (Bazarnovoi et al., 1988). After centrifugation of the samples (1000 rpm, 5 min), the optical density of the supernatant liquid was measured on an SF-26 (Lomo) at a wavelength of 540 nm in a cuvette with an optical path length of 10 mm. The intensity of hemolysis was expressed as a percentage and calculated according to the formula:

$$x = E1 \times 100/Ex,$$

where E1 is the extinction (540 nm) of the supernatant liquid in a test tube with 0.1% NaCl solution and Ex is the extinction (540 nm) of the supernatant liquid of the experimental sample.

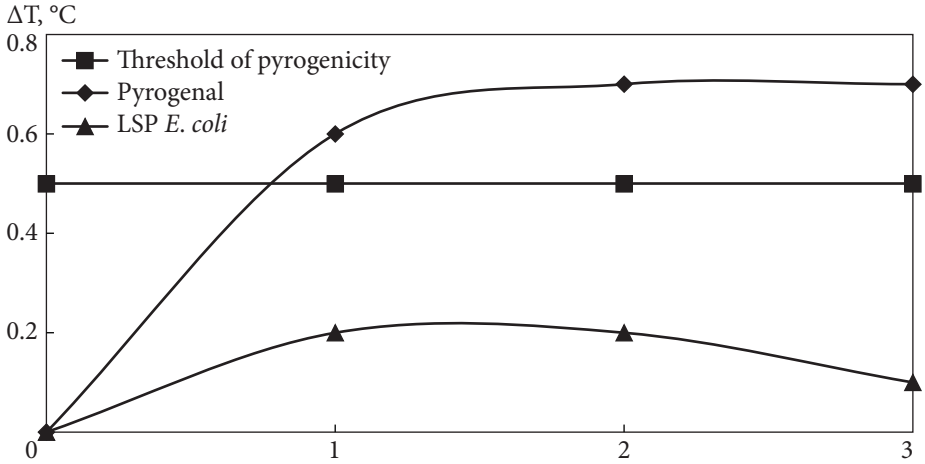

Fig. 3. The pyrogeneity of *E. coli* K LPS

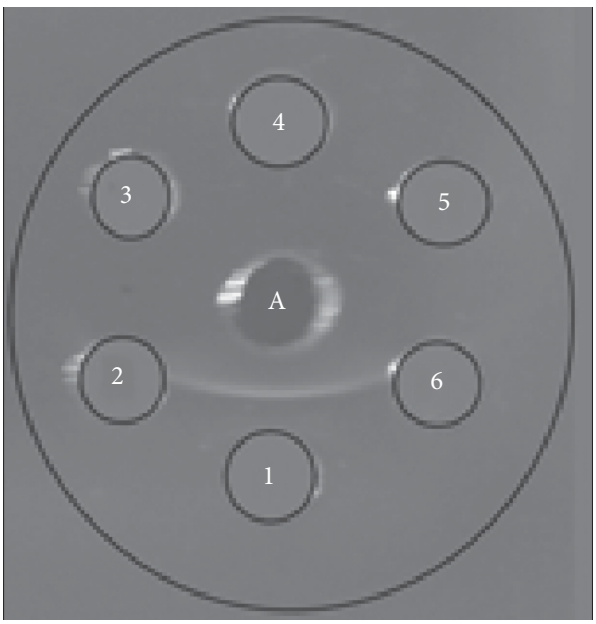

Fig. 4. Double immunodiffusion reaction in agar by Ouchterlony of LPS of *E. coli* K (1); M-17 (2); F-50 (3); L-19 (4); 126 (5); 2890 (6) with O-antiserum (A) against *E. coli* K

StatSoft STATISTICA 10.0.1011 package of statistical programs was used for statistical processing of the research results. Determination of the mean value and errors of the mean (M ± m) was carried out by the parametric method using Student's t-test. Values with $p<0.05$ were considered reliable.

Results. The studied *E. coli* LPS, due to the peculiarities of the water-phenolic extraction method used, was characterized by a rather high content of nucleic acids — 37.4%. Purification of LPS from nucleic acids was carried out by ultracentrifugation (4 h, 105.000 g, 3 times). In purified LPS, 29.46% of carbohydrates, 4.9% of nucleic acids, and 9.53% of proteins were found. The content of 2-keto-3-deoxyoctonic acid (KDO) and heptose, characteristic of LPS components, was 0.03% and 5.64%, respectively. The LPS yield was 23.0%, which was more than 4 times higher than the average values typical for other representatives of *Enterobacteriaceae* (5%—14.0%) (Varbanets et al., 2017). Identification of the monosaccharide composition indicates that galactose dominates in LPS — 70.87%. Fucose, ribose, and glucose were found in smaller quantities (11.86, 10.11, and 7.16%, respectively). The hydroxy acids 2-OH-$C_{12:0}$ — 32.23%, 2-OH-$C_{14:0}$ — 25.07%, and 3-OH-$C_{14:0}$ — 7.26% were identified in the composition of the LPS preparation under study. $C_{14:0}$ — 10.69%, $C_{17:0}$ — 5.35%, $C_{16:0}$ — 3.15%, $C_{15:0}$ — 3.04%, and unidentified fatty acid — 13.21% were also detected. SDS-PAGE electrophoresis (Fig. 2) showed that the studied *E. coli* LPS, similar to the LPS

of the other strains we studied, in particular *E. coli* 126 LPS, is represented by a heterogeneous population, which includes two main types of molecules: high-molecular-weight S-LPS with O-chains of various lengths and low-molecular-weight R-LPS, which does not contain O-specific polysaccharide chains.

The pyrogenicity of the studied LPS was tested under vivarium conditions. Two rabbits weighing between 2.0 and 3.5 kg were used. To conduct a comparative assessment of the pyrogenic characteristics of the studied LPS when administered intravenously to rabbits in a series of dilutions, the minimum pyrogenic dose was established, which was 7.5×10^{-3} μg LPS/mL of non-pyrogenic isotonic solution. One rabbit was injected with sterile saline as a control. Rectal temperature was measured within 3 hours after LPS administration. The initial rectal temperatures of the two rabbits were 38.6 °C and 38.8 °C. The thermometry results (Fig. 3) indicate that an hour after LPS administration, an increase in rectal temperature of 0.2 °C was observed. After 3 hours, the rabbit's temperature did not cross the pyrogenicity threshold. The tested preparation of *E. coli* K LPS turned out to be pyrogen-free: it did not reach either the level of pyrogenicity or the level of «Pyrogenal» (pharmaceutical drug), the active component of which is *Shigella typhi* LPS.

In double immunodiffusion in Ouchterlony agar, LPS of the studied *E. coli* strain in a homologous system exhibited antigen activity (Fig. 4). No cross-serological reactions have been established between the antiserum to heated *E. coli* K cells and LPS of the other *E. coli* strains we studied previously (Varbanets et al., 2012, 2017), namely M-17, F-50, L-19,126, and 2890, which indicates that they do not have common antigenic determinants, that is, belong to different serogroups.

To study the effect of donor erythrocytes on the osmotic resistance, 4 preparations of LPS were taken: native *E. coli* K (1) and *E. coli* 126 (3) and chemically modified by succinylation of *E. coli* K (2) and *E. coli* 126 (4). The results of studies on the determination of osmotic resistance of erythrocytes are presented in Table 1.

The obtained results show that a high percentage of hemolysis is observed in the erythrocytes of all donors after treatment with solutions of LPS preparations, both native and modified. The effect of chemical modification of LPS by

Table 1. **Indicators of osmotic resistance of erythrocytes of practically healthy patients (donors) incubated with LPS (%)**

No. of blood sample	LPS							
	E. coli K		*E. coli* K succin.		*E. coli 126*		*E. coli* 126 succin.	
	A	B	A	B	A	B	A	B
1	20 ± 2.3	95 ± 8.7*	20 ± 2.3	97 ± 10.2*	20 ± 2.3	100 ± 9.7*	20 ± 2.3	98 ± 7.5*
2	27 ± 3.0	93 ± 7.8*	27 ± 3.0	90 ± 8.9*	27 ± 3.0	96 ± 7.9*	27 ± 3.0	98 ± 8.9*
3	23 ± 4.2	92 ± 9.3*	23 ± 4.2	95 ± 8.6*	23 ± 4.2	95 ± 8.5*	23 ± 4.2	98 ± 10.4*
4	25 ± 4.8	95 ± 7.6*	25 ± 4.8	98 ± 10.4*	25 ± 4.8	100 ± 10.5*	25 ± 4.8	95 ± 8.7*
5	25 ± 3.4	95 ± 8.7*	25 ± 3.4	90 ± 8.7*	25 ± 3.4	100 ± 11.0*	25 ± 3.4	98 ± 9.5*
6	30 ± 5.0	95 ± 10.2*	30 ± 5.0	97 ± 9.5*	30 ± 5.0	100 ± 10.8*	30 ± 5.0	98 ± 8.7*
7	23 ± 4.5	92 ± 9.2*	23 ± 4.5	95 ± 9.3*	23 ± 4.5	96 ± 8.4*	23 ± 4.5	92 ± 7.5*
8	25 ± 3.6	93 ± 8.6*	25 ± 3.6	90 ± 7.9	25 ± 3.6	90 ± 7.9*	25 ± 3.6	97 ± 8.2*

A — The percentage of hemolysis (control); B — The percentage of hemolysis (experiment), *— the difference is probable compared to the control ($p<0.05$).

succinylation, which led to a change in the structure of the lipid A part of the LPS molecule, on the ability to influence erythrocyte hemolysis was not noted. This may indicate that some other features of LPS, not established by us, are important in this case.

Discussion. LPS of *E. coli* K isolated from chestnut leaves differs little in its monosaccharide and fatty acid composition from the previously studied (Varbanets et al., 2012, 2017) LPS of other *E. coli* strains isolated from different sources. The difference of the studied strain is an extremely high yield of LPS, as well as its apyrogenic effect, since most *Enterobacteriaceae* LPS are characterized by a small yield of LPS and significant pyrogenicity (Varbanets et al., 2012). As for the LPS of *P. putida*, which was also isolated from the gut of the caterpillar of the chestnut leafhopper moth described by us previously (Brovarska et al., 2020), like the LPS of *E. coli* K, it was apyrogenic and contained two hydroxy acids: 2-OH-$C_{12:0}$ and 3-OH-$C_{12:0}$. But it differed from the LPS of *E. coli* K by a much lower yield and contained a much larger amount of carbohydrates (63.2%). It is known that natural bacterial strains synthesize S-type LPS, which in PAAG electrophoresis form a large number of lines with different degrees of electrophoretic mobility, which corresponds to unsubstituted core oligosaccharides, as well as core oligosaccharides substituted with different numbers of O-specific polysaccharide chains from one to 40. Heterogeneity of slowly moving components can be explained by different lengths of O-specific chains: mobility decreases with the addition of one repeating chain. Like the LPS of other *E. coli* strains, the LPS of the studied strain showed a bimodal distribution typical for S-forms of LPS, which is confirmed by the formation of a classical profile in the form of a ladder on the electrophoretogram. This kind of heterogeneity of LPS is due to the peculiarities of its biosynthesis and can contribute to a denser packing of LPS molecules, which creates a certain topography of the cell surface, necessary for the functioning of the bacterial cell.

Serogrouping of different strains of *Enterobacteriaceae* is the most studied. So, more than 180 serogroups have been described only in representatives of *E. coli*. Whether this culture is a representative of a new serogroup or of those already established today, we do not know. We can only note that the LPS of the studied strain exhibits antigen activity in the homologous system and does not interact with antisera obtained by us against previously studied strains of *E. coli*.

Every year, the number of works testifying to the participation of LPS in various biological processes is increasing, in particular, it is known that LPS have an affinity for erythrocytes and, when adsorbed on their membranes, change the properties of the latter, in particular, their indicator such as resistance to osmotic lysis. The ORE depends on a number of factors: the degree of cell maturity, their shape, changes in plasma composition, and the pathological state of the body. Old erythrocytes activate autoimmune reactions, while young ones suppress them. With hereditary or acquired structural abnormalities of the cytoplasmic membrane, a decrease in the ORE is observed. Such erythrocytes acquire a spherical shape, which indicates that they have entered the phase of completion of their life cycle. Less mature erythrocytes that have just entered the bloodstream from the bone marrow are more osmotically stable. The results obtained by us indicate that the studied LPS of *E. coli* K causes their hemolysis. Similar results were obtained by us earlier (Varbanets et al., 2020.) when studying the effect of LPS of other representatives of *Enterobacteriaceae*, in particular *Pantoea agglomerans* isolated from various plants, on erythrocytes.

Since the LPS of both strains, native and modified, showed almost the same effect, it is likely that both the monosaccharide and fatty acid compositions of LPS does not influence their effect. It is likely that some other properties of LPS affect this process, but the mechanism of this ac-

tion is still unknown. According to the method of determining the osmotic stability of erythrocytes in these experiments, fresh blood was used, which has complement components in its composition. The complement system is an important part of the innate immune system with several membrane-bound and soluble components. In the complement system, there are three separate pathways of the enzymatic cascade: classical, alternative, and lectin (mannose) (Brodszki et al., 2020; Kholodna, 2017). Since LPS are the main antigenic components of the outer membrane of gram-negative bacteria, we can assume their ability to stimulate the activation of the complement system by the lectin pathway (O'Hara et al., 2001; Ali et al., 2023), and this activation leads to the formation of a membrane-attacking complement complex on the membranes of target cells, which leads to their lysis. Inhibition of the lectin pathway of the complement system can reverse the adverse effects of LPS, which has been shown in a model of LPS-induced lung injury in mice and acute respiratory distress syndrome (ARDS) in humans (Ali et al., 2023). Further research is needed to confirm this mechanism.

Thus, if a lot of LPS are accumulated in the body, which causes hemolysis of erythrocytes, then this is a particularly dangerous factor for human health, as it can cause a chain reaction in the development of anemia. Therefore, determining the effect of LPS on the resistance of erythrocytes to osmotic lysis is important in the diagnosis of hematological and other pathological conditions.

Conclusions. The obtained data indicate a high biological potential of the influence of lipopolysaccharides on the osmotic resistance of erythrocytes, which gives reason to attribute this criterion to possible prognostic factors of the risk of osmotic lysis.

Acknowledgments. The authors are grateful to the staff of the Laboratory of Biological Polymeric Compounds for the analysis of the monosaccharide and fatty acid composition of lipopolysaccharide.

The authors are sincerelys grateful to A.F. Likhanov, Doctor of Biological Sciences (National University of Life and Environmental Sciences of Ukraine) for providing *Escherichia coli* K culture for the study.

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Received 11.03.2024

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ХАРАКТЕРИСТИКА ЛІПОПОЛІСАХАРИДУ *ESCHERICHIA COLI* К

Автори виділили з кишечника гусениць каштанової молі дві культури бактерій: *Pseudomonas putida* і *Escherichia coli*. Оскільки у взаємодії з рослинами або живими істотами беруть участь ліпополісахариди, раніше ми охарактеризували ліпополісахарид одного зі штамів — *Pseudomonas putida*. **Метою** даного дослідження була характеристика ліпополісахариду нового штаму *Escherichia coli* K, зокрема його складу та біологічної активності. **Методи.** Ліпополісахарид (ЛПС) отримували з клітин водно-фенольною екстракцією, гетерогенність визначали електрофорезом SDS-PAAG, моносахаридний і жирнокислотний склад визначали хромато-мас-спектрометрією, серологічну активність — імунодифузією в агарі. Для визначення осмотичної резистентності еритроцитів використовували гепаринізовану кров згідно з дослідженнями Базарнової та ін. **Результати.** В очищеному ЛПС виявлено 29.46 % вуглеводів, 4.9 % нуклеїнових кислот і 9.53 % білків. Вміст характерних для ЛПС компонентів 2-кето-3-дезоксиоктонової кислоти і гептози становив відповідно 0.03 % і 5.64 %. Вивчення моносахаридного складу вказує на те, що в ЛПС переважає галактоза — 70.87 %. У менших кількостях виявлено фруктозу, рибозу та глюкозу (відповідно 11.86, 10.11, 7.16 %). У складі досліджуваного препарату ЛПС були гідроксикислоти 2-OH-$C_{12:0}$ — 32.23 %, 2-OH-$C_{14:0}$ — 25,07 % та 3-OH- $C_{14:0}$ — 7.26 %. Також виявлено $C_{14:0}$ — 10.69 %, $C_{17:0}$ — 5.35 %, $C_{16:0}$ — 3.15 %, $C_{15:0}$ — 3.04 % і неідентифіковану жирну кислоту — 13.21 %. Електрофорез SDS-PAGE показав, що досліджуваний ЛПС *E. coli* представлений гетерогенною популяцією, яка містить два основних типи молекул: високомолекулярний S-ЛПС з О-ланцюгами різної довжини та низькомолекулярний R-ЛПС, який не містить О-специфічних полісахаридних ланцюгів. Досліджуваний препарат ЛПС *E. coli* виявився апірогенним: він не досяг ні рівня пірогенності, ні рівня «Пірогеналу» (фармпрепарату), діючим компонентом якого є ЛПС *Shigella typhi*. Визначення осмотичної резистентності еритроцитів показало, що в еритроцитах усіх донорів після обробки розчинами препаратів ЛПС, як нативних, так і модифікованих, спостерігається високий відсоток гемолізу. **Висновки.** Отримані дані свідчать про високий біологічний потенціал впливу ЛПС на осматичну резистентність еритроцитів, що дає підставу віднести цей критерій до можливих прогностичних факторів ризику виникнення осмотичного лізісу.

Ключові слова: *Escherichia coli K, ліпополісахарид, моносахарид, жирнокислотний склад, пірогенність, гетерогенність, осмотична резистентність еритроцитів.*