https://doi.org/10.15407/microbiolj84.06.016

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CHARACTERISTICS OF *LELLIOTTIA NIMIPRESSURALIS* F9A1 LIPOPOLYSACCHARIDE OBTAINED BY DIFFERENT METHODS

Bacterial wetwood, bacterial dropsy, or bacterial slime is a common disease caused by Lelliottia nimipressuralis, which affects the central core of many conifers and deciduous trees. Representatives of this species have been isolated from a variety of trees showing symptoms of the disease, as well as from water and, less commonly, from clinical samples. Important aspects of pathogenesis is the process of pathogen recognition and the protection mechanisms of bacterial cells from plant's antimicrobial substances. It is known that lipopolysaccharides (LPS) take an active part in these processes. They provide the barrier function of the outer membrane, helping to protect bacteria from plant antimicrobial compounds, and the attachment of bacteria to plant cells. Therefore, ***the aim of the work*** *was to study the peculiarities of the chemical composition and functional and biological characteristics of Lelliottia nimipressuralis F9a1 LPS obtained by different methods.* ***Methods.*** *LPS was isolated from dry bacterial mass by phenol-water method (LPS I), extraction method with 0.85% NaCl solution (LPS II), and phenol-water extraction of LPS insoluble in NaCl solution (LPS III). The carbohydrates were analyzed by Dubois method, nucleic acids – by Spirin, protein content – by Lowry and 2-keto-3-deoxyoctonic acid (KDO) – by Osborn. The identification of monosaccharides and fatty acids in LPS preparations was carried out on an Agilent 6890N/5973 inert chromato-mass spectrometry system. The pyrogenicity of LPS was tested keeping the rules of bioethics in rabbits. Serological studies were performed by the Ouchterlony method.* ***Results.*** *LPS II of L. nimipressuralis F9a1 was characterized by low relative yield (2.12%), low content of carbohydrates (9.16%) and nucleic acids (3.7%), and high protein content (26.44%), while the studied preparations of LPS I and LPS III were characterized by a high yield, a rather high content of carbohydrates (46.68 and 38.4%, respectively), an insignificant amount of protein (up to 6.72%) and nucleic acids (up to 4.06%). All LPSs contained up to 0.27% KDO, which is a specific component of the LPS of gram-negative bacteria. The monosaccharide composition indicates that the LPS of the studied L. nimipressuralis strains turned*

Citation: Bulyhina T.V., Pasichnyk L.A., Garkava K.G. Characteristics of *Lelliottia nimipressuralis* F9A1 Lipopolysaccharide Obtained by Different Methods. *Microbiological journal.* 2022 (6). P. 16—25. https://doi.org/10.15407/microbiolj84.06.016

out to be heterogeneous. At the same time, such monosaccharides as fucose, galactose, and glucose were recorded in the LPS of all tested strains. The fatty acid composition of LPS was represented by the presence of fatty acids containing from 12 to 18 carbon atoms. Hydroxylated, saturated, and monounsaturated acids were found. In LPS I and LPS III, the dominant fatty acid was 14:0 (3-OH), which is a kind of marker for the entire family of Enterobacteriaceae. In addition to the marker acid, a 16:1 acid was also predominant in LPS III, whereas in LPS II, 16:1 (32.7%) and 16:0 (22.6%) fatty acids dominated. The pyrogenic effect of L. nimipressuralis LPS studied showed that LPS solutions are pyrogenic. The serological studies showed that tested LPS in homologous systems exhibits antigenic activity. Antisera to L. nimipressuralis F9a1 react with LPS strains IMV 8791, LGK1, and L14b, which may indicate the presence of common antigenic determinants and belonging of these strains to the same serogroup. ***Conclusions.*** *The LPSs of L. nimipressuralis F9a1 were heterogeneous in both monosaccharide and fatty acid composition, which is explained by the use of different methods for their isolation. To isolate LPS from L. nimipressuralis cells, the water-phenol method is better than sodium chloride extraction* since *with using the latter, the LPS yield is very low and very contaminated with proteins. At the same time, the isolation method does not affect the serological activity of the studied LPS. The results received during these biological-functional studies of L. nimipressuralis LPS contribute to the biological characteristics of this species.*

Keywords: *Lelliottia nimipressuralis, lipopolysaccharides, monosaccharide and fatty acid composition, serological and pyrogenic activities.*

As a result of the negative impact of complex factors of abiotic and biotic origin, the phytosanitary state of forests has deteriorated sharply. This process is acquiring features of a global character. The etiology, symptoms, and bacterial pathogenesis of forest woody plants are diverse. The literature contains data on various species and genera of phytopathogenic bacteria that are the causative agents of a common and harmful disease — bacterial wetwood, among which one of the pathogens is *Lelliottia nimipressuralis* (synonyms *Enterobacter nimipressuralis* and *Erwinia nimipressuralis*) [1]. Currently, this species is considered to be one of the main pathogens of bacterial wetwood of forest woody plants in Ukraine [2, 3].

The phytopathogenic polybiotrophic bacterium *L. nimipressuralis* infects many species of both shade and forest trees. But with the normal growth and development of the tree, it performs useful functions. When the vital state of trees is disturbed, such bacteria become a powerful endogenous vector for the occurrence of diseases — epiphytotic vascular (for forest trees) and tracheid (for shade trees).

At present, bacteriosis of forest woody plants and their pathogens have not been studied properly, although in recent decades there has been increased attention to this problem [4—6]. Research should be aimed at deepening the study of bacteriosis in order to elucidate the complex relationships between representatives of various systematic and functional groups of microorganisms in the infectious pathology of forest woody plants.

As known, important aspects of pathogenesis are the pathogen recognition process and the mechanisms of bacterial cell protection from plant's antimicrobial substances. These processes are actively involved in lipopolysaccharides (LPS), which are the key structure that ensures the establishment and development of association with a partner plant. They are not only of fundamental importance for the viability of bacteria but also make a significant contribution, both directly and indirectly, to many aspects of plant-microorganism interactions [7—9].

LPS provide an outer membrane barrier function, helping to protect bacteria from plant antimicrobial compounds, as well as bacterial attachment to plant cells. This fact determines the relevance of further studies of the LPS functional role as a promising direction in elucidating the molecular mechanisms of the bacterial pathogenesis. Therefore, **the purpose of the work** was to study the peculiarities of the chemical composition, functional and biological characteristics of *Lelliottia nimipressuralis* LPS obtained by different methods.

Materials and methods. The object of the study was strain of *L. nimipressuralis* F9a1 isolated from affected common oak (State Enterprise «Fastivske Forestry» in the Kyiv region) in 2020. Bacteria were grown on potato agar for 36 h at 28—30 °C. After cultivation, the cells were collected by centrifugation (20 min, 5000 g), washed with saline, and dried by treatment with acetone (twice) and ether (once).

Lipopolysaccharide LPS I was extracted from dried cells with a 45% aqueous phenol solution at 65—68 °C. The resulting aqueous fractions were dialyzed against tap and then distilled water to remove phenol [10]. LPS was purified from nucleic acids by ultracentrifugation (104.000 g, 4 h).

The method of extraction of LPS with a weak solution of sodium chloride [11] makes it possible to isolate the native O-antigenic complex. 10 g of raw bacterial mass was suspended in NaCl for 3—4 h on a mechanical (magnetic) mixer at room temperature or overnight at 4 °C (extraction was carried out 3—5 times). Each suspension was centrifuged at 5000 g for 30—40 min. After that, the combined extracts were dialyzed against distilled water, centrifuged, and freeze-dried (LPS II). The residues were extracted by water-phenol method as described above (LPS III).

The amount of neutral carbohydrates was determined by the Dubois method [12]. The results were evaluated by the color change during the reaction of phenol with sulfuric acid on a spectrophotometer SF-26 at 490 nm. The carbohydrate content was determined according to standard calibration curves pre-built for glucose. The content of nucleic acids was assessed by the method of Spirin [13], proteins – by Lowry using Folin's reagent [14], and 2-keto-3-deoxyoctonic acid (KDO) – by reaction with thiobarbituric acid [15].

Neutral monosaccharides were identified after hydrolysis of preparations in 2 M $C_2HF_3O_2$ (6 h, 100 °C). Monosaccharides were analyzed as polyol acetates [16] 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. They were identified by comparing the retention time of polyol acetates of the studied samples with standards, as well as using the ChemStation computer database. Quantitative ratios of individual monosaccharides were expressed as% to the total sum of peak areas.

The fatty acid composition was determined after sample hydrolysis in a 1.5% acetyl chloride solution in methanol (100 °C, 4 h), and fatty acid methyl esters were analyzed on an Agilent 6890N/5973 inert chromatography-mass spectrometric system with an HP-5MS column (30 m×0.25 mm× ×0.25 μm); the carrier gas was helium at a flow rate of 1.2 mL/min. Fatty acids were identified using a personal computer database and a standard mixture of fatty acid methyl esters. The quantitative ratios of individual fatty acids were expressed as % to the total sum of peak areas [11].

The results of the study of monosaccharide and fatty acid compositions were obtained using the equipment for research work of the Center for Collective Use at the D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine.

Pyrogenicity was studied on rabbits (weighing up to 3.5 kg) by intravenous injection of the minimum pyrogenic dose of $7.5 \cdot 10^{-3}$ μg/mL, which was established in a series of LPS dilutions, followed by animal thermometry for 3 h. LPS was considered non-pyrogenic if the sum of the temperature increase for three rabbits was less than or equal to 1.4 °C; if it exceeded 2.2 °C, LPS was considered pyrogenic [17]. The work was conducted in accordance with the «General Ethical Principles of Animal Experiments».

O-antiserum was obtained against heated (2.5 h, boiling water bath) *L. nimipressuralis* cells. Rabbits were immunized intravenously five times, with an interval of 4 days; the cell concentration was $2 \cdot 10^9$/mL (from 0.1 to 1 mL). The antigenic activity of LPS was studied by double immunodiffusion in agar according to Ouchterlony [18]. As an antigen, we used LPS *L. nimipres-*

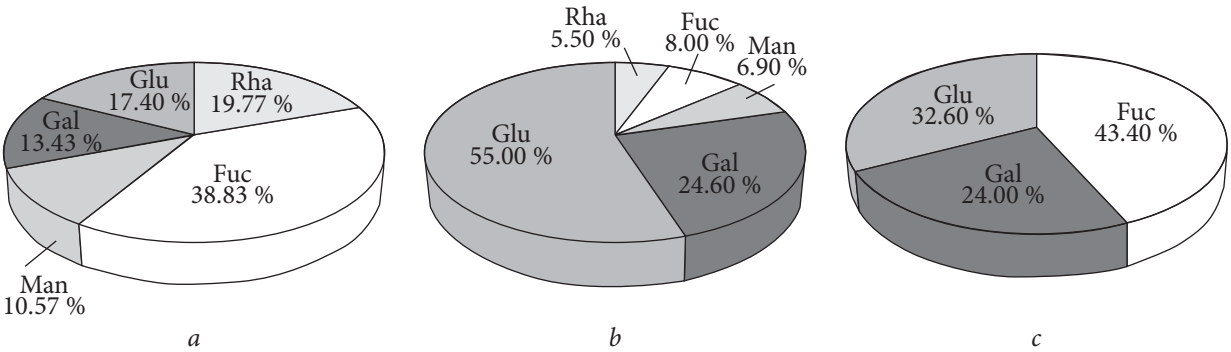

Fig. 1. Monosaccharide composition (% of the total sum of peak areas) of *L. nimipressuralis* LPS: *a* — LPS I; *b* — LPS II; *c* — LPS III

suralis F9a1 extracted by different methods, as well as LPS of other strains of this species (IMV 8791 (collection strain of the department of phytopathogenic bacteria), LGK1 (isolated from affected white fir, State Enterprise «Kutske Forestry» in the Ivano-Frankivsk, region in 2019), and L14b (isolated from affected oak, State Enterprise «Fastivske Forestry» in the Kyiv region, in 2018), isolated by us using phenol-water methods.

Statistical analysis of the data obtained was carried out using the current statistical methods, as well as the Excel 2000 computer program.

Results. The *L. nimipressuralis* F9a1 LPSs isolated from dry bacterial mass by different methods were characterized by different relative yields, from 2.12 to 11.69% (Table 1): the lowest yield was observed for LPS II and the highest — for LPS III. In the studied preparations of LPS I and LPS III, a rather high content of carbohydrates was determined — 46.68 and 38.4%, respectively, along with an insignificant amount of protein (up to 6.72%) and nucleic acids (up to 4.06%). At the same time, LPS II was characterized by a low content of carbohydrates (9.16%) and nucleic acids (3.7%) (Table 1), as well as a high content of protein (26.44%). All LPS, independently of their bacterial origin, contained at least one KDO residue or its derivative. The content of KDO, a specific LPS component of gram-negative bacteria, in the studied preparations ranged from 0.068 (LPS II) to 0.27% (LPS III).

The chromatography-mass spectra of alditol acetates (Fig. 1) revealed rhamnose, fucose, mannose, glucose, and galactose as components of the carbohydrate moiety of LPS I and LPS II and fucose, glucose, and galactose for LPS III. The dominant monosaccharides in LPS I, LPS II, and LPS III were: fucose (38.83%); glucose (55%); and fucose (43.4%), glucose (32.6%), and galactose (24%), respectively. The percentage of other monosaccharides was significantly lower. The LPS I and LPS II preparations differed only in the quantitative content of monosaccharides. While LPS III differed from the other two preparations not only in quantitative but also in qualitative composition.

The structure of lipid A, the endotoxic center of LPS, influences its immunomodulatory properties. The study of the fatty acid composition of LPS showed the presence of fatty acids

Table 1. **Chemical composition of *Lelliottia nimipressuralis* F9a1 LPS**

Preparation	Yield of LPS (% of dry mass of cells)	Content (% of dry mass of LPS)			
		Carbohydrates	Protein	NA*	KDO**
LPS I	10.09	46.68	traces	4.06	0.22
LPS II	2.12	9.16	26.44	3.7	0.068
LPS III	11.69	38.4	6.72	1.63	0.27

* NA — nucleic acids; ** KDO — 2-keto-3-deoxyoctonic acid.

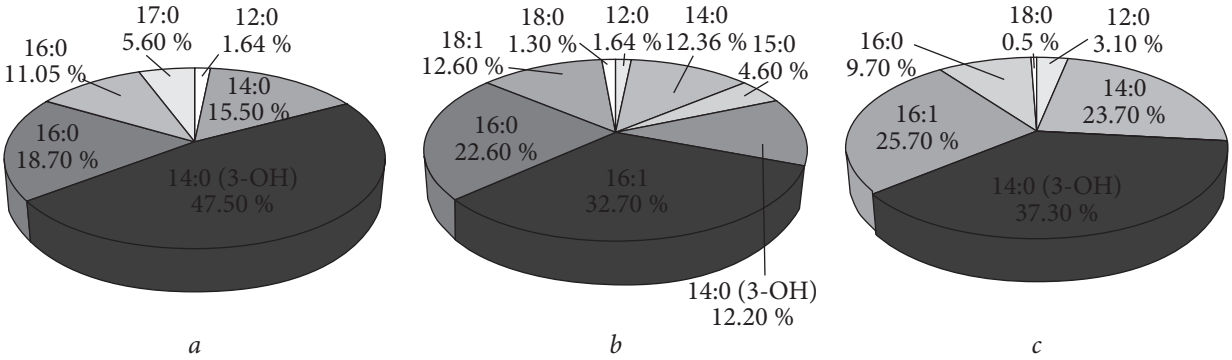

Fig. 2. Fatty acid composition (% of the total sum of peak areas) of *L. nimipressuralis* F9a1 LPS: *a* — LPS I; *b* — LPS II; *c* — LPS III

containing from 12 to 18 carbon atoms in the chain. The dominant fatty acid in *L. nimipressuralis* F9a1 LPS I and LPS III was 14:0 (3-OH), which is a kind of marker for the entire family of *Enterobacteriaceae*. In addition to the marker acid, 16:1 (25.7%) acid was also dominant in the LPS III preparation. Also, a significant amount of 14:0 (23.7%) acid was identified in the LPS III, and in the LPS I, 14:0 (15.5%), 16:1 (18.7%), and 16:0 (11.05%) acids were identified. In LPS II, 16:1 (32.7%) and 16:0 (22.6%) acids were dominant, and 14:0 (12.36%), 14:0 (3-OH) (12.2%), and 18:1 (12.6%) acids were identified in significant amounts. Other fatty acids were found in much smaller amounts. Lipids A of all studied LPS differed in the qualitative and quantitative composition of fatty acids.

It is known that LPSs of gram-negative bacteria, regardless of the source of their isolation, are characterized by endotoxicity, in particular pyrogenicity and toxicity. The studies of the pyrogenic effect of LPS were carried out according to the biological method of qualitative control regarding the presence of bacterial endotoxins in medical preparations. For a comparative definition of the pyrogenic characteristics of the tested LPS, the minimum pyrogenic dose was established, which was 7.5 10^{-3} μg LPS/mL of a non-pyrogenic isotonic solution.

The thermometry results (Fig. 3) showed that the *L. nimipressuralis* F9a1 LPS solution caused an increase temperature in experimental animals by more than 0.5°C (which is beyond the physiological norm of healthy animals). Within the first hour after LPS administration, a sharp increase in animal's temperature was observed. Within the second hour, a slight decrease in temperature was established, and at 3 h, an increase in temperature was again observed. According to the manifestation of the pyrogenic effect with intravenous injection of the minimum pyrogenic dose, the studied strain was similar to «Pyrogenal», a pharmaceutical preparation whose active ingredient is *Shigella typhi* LPS.

It is known that LPS is the main antigen of gram-negative bacteria. To study its immunochemical properties, polyclonal O-antisera were used. It was found that the studied *L. nimipressuralis* F9a1 LPS showed antigen activity in the homologous system by double immunodiffusion reaction in agar according to Ouchterlony. In addition, we have found that antiserum to *L. nimipressuralis* F9a1 reacts with LPS of strains IMV 8791, LGK1, and L14b, as well as with LPS of strain F9a1, which were isolated by different methods. This indicates that they have common antigenic determinants and that all of these strains belong to the same serogroup. It can also be seen that the isolation method did not affect the serological activity of the studied LPS.

Discussion. Bacterial wetwood disease of plants occurs when bacterial pathogens enter

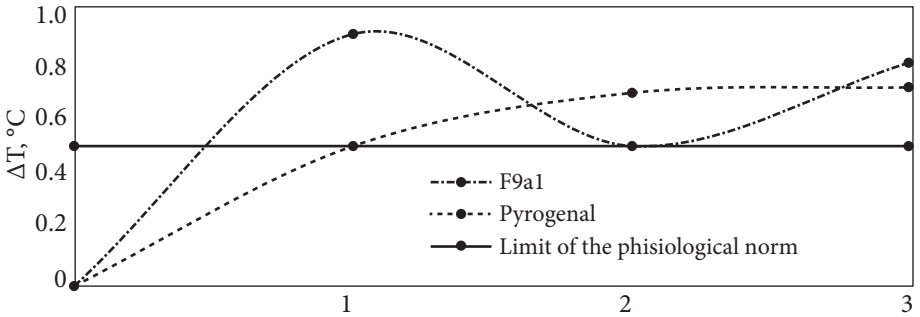

Fig. 3. Pyrogenicity of *L. nimipressuralis* F9a1 LPS

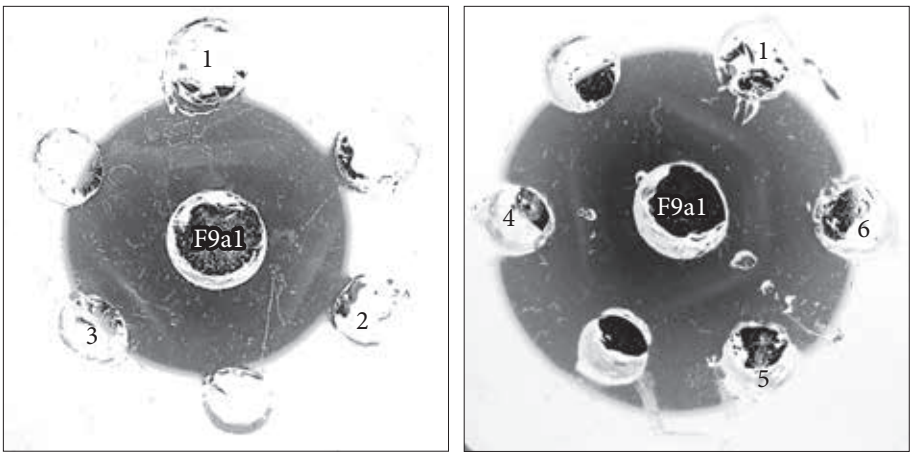

Fig. 4. The reaction of double immunodiffusion in agar by Ouchterlony of O-antiserum against *L. nimipressuralis* F9a1 (central holes) and LPS from *L. nimipressuralis* F9a1 ((LPS I (1), LPS II (2), and LPS III (3)), IMV 8791 (4), LGK1 (5), and L14b (6). The white bars between the holes indicate the presence of antigen-antibody complexes

through damage and multiply in branches and trunks. It was originally studied by Carter (1945), and *L. nimipressuralis* was described as the sole causative agent of the disease [19]. As mentioned above, LPSs, glycopolymers of cell membranes of gram-negative bacteria, are actively involved in processes of pathogenesis. LPSs have been the subject of intensive research for more than half a century [20—22]. They are glycolipids with carbohydrate sequences unique to each species and serotype of bacteria. In addition, LPS is an endotoxin and a major stimulator of innate immune cells in mammals, which makes it an ideal candidate for early pathogen detection. Until now, no report on the the structure and chemical composition of the *L. nimipressuralis* LPS has been found in the literature. In this context, studies of structural features, chemical composition, serological and biological activity of *L. nimipressuralis* LPS are relevant.

LPS preparations of *L. nimipressuralis* were isolated from dry bacterial mass by phenol-water method (LPS I), extraction method with 0.85% NaCl solution (LPS II), and phenol-water extraction of LPS insoluble in NaCl solution (LPS III). Purified LPSs were characterized by different relative yields from 2.12 to 11.69%, which for the two preparations exceeds the average values for the other representatives of *Enterobacteriaceae* (5%). LPS isolated by extraction with a weak so-

lution of sodium chloride was characterized by low contents of carbohydrates, KDO, and nucleic acids, as well as a high content of protein. At the same time, LPSs isolated by the other methods were characterized by rather high contents of carbohydrates and KDO along with small amount of protein and nucleic acids.

Galanos et al. [23] pointed out the internal variability of the physicochemical properties and biological activity of LPS extracted by different methods from different bacterial sources. Commonly used extraction methods produce preparations contaminated with other biologically active molecules such as proteins and ribonucleic acids, which was observed for the LPS preparation isolated by extraction with a NaCl solution.

One of the factors responsible for the virulence of gram-negative bacteria is endotoxin, an integral component of their outer membrane. These polymers are characterized by a common structure consisting of three regions: a polysaccharide O-antigen, an oligosaccharide core, and lipid A. The carbohydrate moiety of LPS is the most structurally heterogeneous region affecting bacterial virulence. Its loss can lead to a decrease in the pathogenicity of microorganisms. The O-specific part of LPS consists of repeating oligosaccharide units that are characteristic (by their composition and structure) for a certain type of microorganisms and usually consist of 2—6 and sometimes 7 or 8 monosaccharides forming straight- or branched-chain oligosaccharides.

The chemical structure of LPS of many microorganisms has not been completely studied to date. At the same time, nothing is known about the structure of the carbohydrate part of *L. nimipressuralis* LPS. We have isolated LPSs and studied their monosaccharide composition, analysis of which showed the presence of rhamnose, fucose, mannose, galactose, and glucose in LPS I and LPS II and only fucose, galactose, and glucose in LPS III. The monosaccharide rhamnose is commonly found in many LPSs. For example, there have been found *Salmonella arizonae* O62 [25], *S. enteritidis* [26], *Shigella dysenteriae* [27], and *Pseudomonas syringae* [24, 28]. Three hexoses (mannose, glucose, and galactose), which were present in the polysaccharide chain of the studied LPS *L. nimipressuralis*, can be part of not only the O-antigen but also the outer part of the oligosaccharide core.

Lipid A is the endotoxic center of the LPS molecule [29] and is responsible for its biological effects. The study of the fatty acid composition of Lipid A showed the presence of hydroxy, saturated and monounsaturated acids with a chain length of 12 to 18 carbon atoms. In LPS I and LPS III, the 3-OH-C14:0 acid dominates (47.5 and 37.5%, respectively), which is a kind of marker for the whole family of *Enterobacteriaceae*. Whereas, in LPS extracted with NaCl solution, 16:1 and 16:0 acids were dominant. LPS extracted with NaCl was characterized by an insignificant yield and fatty acids contaminating it, which are not part of the lipid part of LPS, but are components of the cell membrane, as shown by the researchers [1]. Small amounts of nonspecific for lipid A fatty acids 12:0 (1.64—3.1% in all studied LPS), 15:0 (4.6% in LPS II), 17:0 (5.6% in LPS I) and 18:0 (1.3 and 0.5% in LPS I and LPS III, respectively) were found. Probably, these fatty acids are not included in the composition of lipid A of the studied strain, and their presence in the analyzed samples is the result of their strong connection with LPS.

LPS serves as a biomarker to help distinguish gram-negative bacteria serologically. The double immunodiffusion reaction in agar according to Ouchterlony established that the studied LPS of *L. nimipressuralis* F9a1 in the homologous system showed antigen activity. It was found that antiserum to *L. nimipressuralis* F9a1 reacts with LPS strains IMV 8791, LGK1 and L14b of this species. This indicates that they have common antigenic determinants and that these strains belong to the same serogroup. Although LPS were isolated from different regions

and host plants, they were serologically similar. The method of isolation of LPS from the bacterial mass did not affect the serological activity. These data can be used to develop a serological classification scheme.

Thus, the LPS of *L. nimipressuralis* F9a1 were isolated by different methods, purified, and chemically characterized. The studied LPS were heterogeneous in both monosaccharide and fatty acid composition, which are explained by the methods of their isolation. The study of the pyrogenic effect showed that the *L. nimipressuralis* F9a1 LPS solution was more pyrogenic than «Pyrogenal». Antisera to *L. nimipressuralis* F9a1 react with LPS strain strains IMV 8791, LGK1 and L14b of this species, which may indicate their common antigenic determinants and belonging of these strains to the same serogroup. For isolating LPS from *L. nimipressuralis* the water-phenol method is better than sodium chloride extraction. Since, when using the latter method, the LPS yield is very low and very contaminated with protein. The results obtained in the course of biological and functional studies of LPS *L. nimipressuralis* contribute to the study of the biological characteristics of this species.

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Received 28.06.2022

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ХАРАКТЕРИСТИКА ЛІПОПОЛІСАХАРИДУ *LELLIOTTIA NIMIPRESSURALIS* F9A1, ОТРИМАНОГО РІЗНИМИ МЕТОДАМИ

Бактеріальна водянка є поширеною хворобою, спричиненою *Lelliottia nimipressuralis*, яка вражає центральне ядро багатьох хвойних та листяних дерев. Представники цього виду виділяють із різноманітних дерев, що виявляють симптоми цієї хвороби, а також з води та рідше — з клінічних зразків. Одним із важливих аспектів патогенезу є процес розпізнавання збудника та механізми захисту бактеріальної клітини від антимікробних речовин рослини. Відомо, що у цих процесах активну участь беруть ліпополісахариди (ЛПС) — глікополімери клітинних мембран бактерій. Вони забезпечують бар'єрну функцію зовнішньої мембрани, сприяючи захисту бактерій від рослинних антимікробних сполук, а також прикріпленню бактерій до рослинних клітин. Тому **метою роботи** було вивчення особливостей хімічного складу, функціональної та біологічної характеристики ЛПС *Lelliottia nimipressuralis*, отриманих різними методами. **Методи.** ЛПС виділяли із сухої бактеріальної маси фенол-водним методом (ЛПС I), методом екстракції 0.85 % розчином NaCl (ЛПС II) і фенол-водною екстракцією ЛПС, нерозчинного в розчині NaCl (ЛПС III). Кількісний вміст вуглеводів визначали методом Дюбуа; нуклеїнових кислот — Спіріна; білка — Лоурі; 2-кето-3-дезоксиоктонової кислоти (КДО) — Осборна. Метилові ефіри жирних кислот та моносахариди у вигляді ацетатів поліолів

аналізували на хромато-мас-спектрометричній системі Agilent 6890N/5973 inert. Пірогенність досліджували на кролях шляхом внутрішньовенного введення мінімальної пірогенної дози $7{,}5 \cdot 10^{-3}$ мкг/мл з подальшою термометрією тварин протягом 3-х годин. Антигенну активність ЛПС досліджували методом подвійної імунодифузії в агарі за методом Оухтерлоні. **Результати.** ЛПС II *L. nimipressurali* F9a1 характеризувався низьким відносним виходом (2.12 %), низьким вмістом вуглеводів (9.16 %) і нуклеїнових кислот (3.7 %), високим вмістом білка (26.44 %), у той час як досліджувані препарати ЛПС I і ЛПС III характеризувалися високим виходом, досить високим вмістом вуглеводів (відповідно 46.68 та 38.4 %), незначною кількістю білка (до 6.72 %) та нуклеїнових кислот (до 4.06 %). Усі ЛПС містили до 0.27 % КДО, яка є специфічним компонентом ЛПС грамнегативних бактерій. За моносахаридним складом ЛПС досліджених штамів *L.* nimipressuralis виявилися гетерогенними. У той же час такі моносахариди, як фукоза, галактоза і глюкоза, були зареєстровані в ЛПС усіх досліджених штамів. Жирнокислотний склад ЛПС був представлений рядом жирних кислот, що містять від 12 до 18 атомів вуглецю. Виявлено гідроксильовані, насичені і мононенасичені кислоти. У ЛПС I і ЛПС III домінуючою жирною кислотою була 14:0 (3-OH), яка є свого роду маркером для всього сімейства ентеробактерій. Крім маркерної кислоти, у ЛПС III також переважала 16:1 кислота, тоді як для ЛПС II домінуючими жирними кислотами були 16:1 (32.7 %) та 16:0 (22.6 %). Дослідження пірогенної дії ЛПС штамів *L. nimipressuralis* показало, що розчин ЛПС є пірогенним. Серологічні дослідження виявили, що досліджуваний ЛПС у гомологічній системі має антигенну активність. Антисироватка до *L. nimipressuralis* F9a1 реагує із ЛПС штамів IMB 8791, ЛГК1 і L14b, що може вказувати на спільні антигенні детермінанти та приналежність цих штамів до однієї серогрупи. **Висновки.** ЛПС штаму *L. nimipressuralis* F9a1 не однорідні як за моносахаридним, так і за жирнокислотним складом, що пояснюється використанням різних методів їх виділення. Для виділення ЛПС з клітин *L. nimipressuralis* водно-фенольний метод був кращим, ніж екстракція хлоридом натрію, оскільки при використанні останнього методу вихід ЛПС дуже низький і дуже забруднений білком. При цьому спосіб виділення не впливав на серологічну активність досліджуваного штаму ЛПС. Результати, отримані під час біолого-функціональних досліджень ЛПС *L. nimipressuralis*, роблять свій внесок у вивчення біологічних особливостей цього виду.

Ключові слова: *Lelliottia nimipressuralis, ліпополісахариди, моносахаридний та жирнокислотний склад, серологічна та пірогенна активності.*