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EXPERIMENTAL ARTICLES

Characterization of Lipopolysaccharides from *Pseudomonas fluorescens* IMB 2108 (Biovar II) and IMB 2111 (Biovar IV) with O-Chains Represented by α-Glucan

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Abstract—Results of studies of the structurally unique O-chains of lipopolysaccharides, which were isolated from the dry biomass of *Pseudomonas fluorescens* IMB 2108 (biovar II) and IMB 2111 (biovar IV) by the Westphal technique and purified by repeated ultracentrifugation, are reported. The bulk of the lipopolysaccharide preparations contained S- and R-molecules at an average molar ratio of 1 : 2. The main components of the hydrophobic moiety of lipid A were 3-hydroxydecanoic, 2-hydroxydodecanoic, 3-hydroxydodecanoic, dodecanoic, hexadecanoic, and octadecanoic acids, as well as hexadecenoic and octadecenoic acids. Glucosamine and phosphoethanolamine were identified as components of the hydrophilic moiety of lipid A. The degree of lipid A phosphorylation amounted to 3–4%. Fractions of the core oligosaccharide contained glucose, galactose, mannose, rhamnose, arabinose, glucosamine (only in strain IMB 2108), alanine, phosphoethanolamine, phosphorus, and 2-keto-3-deoxyoctulosonic acid (KDO). Heptose was present in trace amounts. O-specific polysaccharide chains were represented by a linear polymer of *D*-glucose units, which were linked together via α-(1,4) glycoside bonds. The existence of *P. fluorescens* strains that have α-1,4-glucan as the O-chain of their lipopolysaccharides has not been described before.

Key words: *Pseudomonas fluorescens*, lipopolysaccharide, lipid A, core oligosaccharide, O-specific polysaccharide, α-1,4-*D*-glucan.

The gram-negative bacterium under consideration is a typical representative of fluorescent bacteria of the genus *Pseudomonas*, which are characterized by phenotypic and genotypic heterogeneity. Based on this circumstance, the latest (9th) edition of *Bergey's Manual* [1] lists five biovars of this species lacking definitive taxonomic assignment. Results of numerical analysis of the structure and properties of the lipopolysaccharides (LPSs) of *P. fluorescens* lends further support to the concept of the heterogeneity of this species [2]. Indeed, the similarity between certain strains representing distinct biovars of the species does not exceed 60%. At the DNA level, the homology of *P. fluorescens* strains belonging to biovar II (=biotype B [3]) and biovar IV (=*Pseudomonas lemonnieri* [3]) is also indicative of the intrinsic heterogeneity of these bacteria [4]. Methods of identification and taxonomical assignment of the species have still not been perfected due to the lack of significant parameters based on which a strain can be assigned to a certain taxon.

LPSs are major components of the outer membrane of the cell wall of gram-negative bacteria. Because of their immediate contact with membrane proteins, LPSs ensure the integrity, stability, and functional competence of the outer membranes of microbial cells. Being located at the outer surface of the membrane, LPSs play an important role in bacterial interactions with the environment and other organisms, e.g., by determining the immunogenic properties of bacteria. LPSs act as endotoxins: their release from bacterial cells that have entered a macroorganism induces an immune response in the latter. LPS biosynthesis is determined by several genes (differing in the extent to which they are conserved); for this reason, data on the structures and properties of these bioactive macromolecules can be used in taxonomy for clarifying phylogenetic interactions between bacteria.

In all the gram-negative bacteria studied thus far, the molecule of an LPS is built according to the same pattern and contains lipid A and a hydrophilic carbohydrate moiety in which an O-specific polysaccharide chain and a core oligosaccharide (the central portion of the LPS molecule) are distinguished. The latter is

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covalently attached to the hydrophobic moiety of the LPS, i.e., lipid A, which links the carbohydrate domain to the outer membrane of the bacterial cell. Unlike lipid A and the core, which are relatively conserved portions of the complex LPS molecule, O-specific polysaccharide chains are structurally diverse and exhibit strain specificity. In the majority of cases, however, their structure follows the same basic principle: any O-specific polysaccharide consists of repeated units containing one to eight (or more) carbohydrate residues that may carry substituents. For example, the O-specific polysaccharides of LPSs in previously studied *P. fluorescens* strains are built from repeated units containing preferentially amino sugars [5, 6]. Individual monosaccharides carry non-carbohydrate substituents. A considerable body of data on the structural and biological properties of LPSs isolated from different species of gram-negative bacteria has been accumulated thus far. Nevertheless, the LPSs of *P. fluorescens* remain largely unknown. The classification of gram-negative bacteria, which is based on the serological properties of O-specific polysaccharide chains of LPSs, has not been developed for *P. fluorescens*.

Earlier, we studied [2–7] the LPSs of several strains of *P. fluorescens*, which represented various biovars of this bacterial species, including that of type strain IMB 4125 (=ATCC 13525), representing biovar I. We elucidated the macromolecular organization of these LPSs and described characteristic features of the structural parts of their macromolecules. We were able to demonstrate that the LPSs isolated from distinct strains of *P. fluorescens* have a similar fatty acid composition and common components in the hydrophilic portion of lipid A. As regards the core composition and the structure of the O-specific polysaccharide chain, the LPSs studied exhibited variable heterogeneity; biovar I was an exceptional case, since it was shown to comprise three strains with identical O-chains [6].

In this study, we report the results of our investigation of LPSs from *P. fluorescens* IMB 2108 (biovar II) and IMB 2111 (biovar IV). We sought to isolate, purify, and cleave the LPSs with a view to obtaining preparations of the structural parts of their macromolecules (lipid A, core oligosaccharide, and O-specific polysaccharide chains). Furthermore, we undertook to (1) reveal characteristic features of the composition and structure of the preparations thus obtained, (2) characterize the serological properties of the LPSs, (3) assess the degree of their similarity to the LPSs of previously studied *P. fluorescens* strains, and (4) use the data generated to clarify the taxonomic position of *P. fluorescens* strains within the biovars and in general.

MATERIALS AND METHODS

Under study were *Pseudomonas fluorescens* strains IMB 2108 (biovar II) and IMB 2111 (biovar IV), which we obtained from the collection of microorganisms maintained at the Zabolotnyi Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine. The bacteria were grown at 28°C on meat–peptone agar (MPA) for 24 h. The bacterial mass was washed off the agar surface with physiological saline, and the cell suspension was centrifuged at 6000 rpm for 30 min. The resulting pellet was washed with physiological saline and desiccated with acetone and diethyl ether. O-specific sera were obtained by immunizing rabbits with suspensions of microbial cells killed by heating at 100°C for 2.5 h (the concentration of bacteria in each suspension was set to 4×10^9cells/ml). Serological studies were performed using published methods [6, 8]. The LPS was isolated from the dry biomass by phenol–water extraction [9] at 65°C and purified by repeated ultracentrifugation (three periods at 105000 *g* of 4 h each). The pellet was lyophilized. In order to obtain fractions containing lipid A, core oligosaccharide, and O-specific polysaccharide, the LPS preparation was subjected by acidic hydrolysis under mild conditions (1% acetic acid at 100°C for 1.5 h). The water-insoluble lipid A fraction was separated by centrifugation at 6000 rpm. Fractions corresponding to core oligosaccharide and the O-specific chain were isolated by gel filtration of the carbohydrate portion of the LPS on a column packed with Sephadex G-50 and equilibrated with 0.025 M pyridine–acetate buffer (pH 4.5). Fatty acids contained in lipid A were methylated in sealed ampoules containing 1.5 M HCl in methanol (at 100°C for 3 h). Analysis of the fatty acid methyl esters was performed on a Chrom-5 gas chromatograph (Czech Republic) using columns of two types: (1) 1200×3 mm with 5% SE-30 on Chromaton N-AW-DMCS or (2) 2000×3 mm with 5% DEGS-PS on Supelcoport (100–120 mesh). The chromatograph was equipped with a flame-ionization detector; helium served as a carrier gas. Trifluoroacetylation of the methyl esters of hydroxy acids was performed as described in [8]. The derivatives obtained were analyzed on a gas chromatograph under the same conditions. KDO was determined by a reaction with thiobarbituric acid [10]. The total carbohydrates and phosphorus were measured in the LPS preparations using published protocols [5]. Neutral sugars were determined in hydrolysates (2 N HCl at 100°C for 4 h) of the carbohydrate moiety of the LPS by two techniques: (1) ion-exchange chromatography on a column packed with the anion exchanger resin BTA 2118 (Biotronik, Germany) in a system of discontinuous gradient of potassium-borate buffers [5], and (2) gas–liquid chromatography [8]; in the latter case, the sugars were measured in the form of polyol acetates. The content of glucosamine, galactosamine, alanine, and other amino components was determined on a Biotronik amino acid analyzer using a discontinuous gradient of sodium-citrate buffers. HPLC was used for rechromatography of the preparations of O-specific polysaccharides [5]. The absolute configuration of glucose was determined by gas–liquid chromatography following derivatization of the hydrolysis products of O-specific polysaccharides to acetylated (*R*)-2-octyl

glycosides [11]. NMR spectra were recorded on a Bruker DRX-500 spectroscope (D_2O) at 45°C. Acetone (δ_H 2.225; δ_C 31.45) was used as an internal standard. Spectrophotometric analysis of the LPS preparations was performed using a DU-8B Beckman spectrophotometer (United States).

RESULTS AND DISCUSSION

The heterogeneity of the previously studied *P. fluorescens* strains [5–7] correlated with differences in the structure of the O-chains of their LPSs in strains belonging to different biovars. Biovar I was an exceptional case, since it comprised three strains with identical O-chain structures. Therefore, we viewed the identity of the O-chain structures of LPSs from two strains of different biovars of *P. fluorescens* as an extraordinary feature justifying comparative study of the LPSs in these strains.

The LPS preparations isolated from *P. fluorescens* IMB 2108 (biovar II) and IMB 2111 (biovar IV) by hot aqueous phenol and purified by ultracentrifugation showed little, if any, differences when studied by spectrophotometric scanning (solutions of 1 mg/ml were studied). An absorption band in the range 200–220 nm predominated in the spectra. The band corresponding to the absorption of proteinaceous components corresponded to a 3–4% protein content. Nucleic acids were present in trace amounts. The LPS preparations contained 46% carbohydrates (judging by the reaction with phenol and sulfuric acid); their degree of phosphorylation did not exceed 4%. The yield of LPS preparations amounted to 4% of the dry weight of acetone extract of the biomass of the bacterial strains studied.

The LPS preparations were active in serological reactions with homologous O-antisera (obtained by immunization of rabbits with bacterial cells killed by heating), as was shown by a double immunodiffusion in agar (two lines) and precipitation (titer, 1 : 600000). The preparations under study were also active in heterologous reactions (the appearance of one line in the reaction of double immunodiffusion in agar and a titer of 1 : 100000 in the reaction of precipitation), which indicated that the O-specific carbohydrate chains contained homologous regions. The LPSs under study showed no activity in cross-reactions of O-antisera as compared to the neotype strain *P. fluorescens* IMB 4125 and strains representing other biovars of the species.

In order to isolate preparations of other structural portions of the molecule, the LPSs under study were subjected to mild hydrolysis, which cleaves the acid-labile bond between KDO residues and glucosamine (components of the core oligosaccharide and lipid A, respectively). Water-insoluble lipid A fractions, isolated after degradation of the original LPS preparations with 1% acetic acid (at 100°C for 1.5 h), accounted for 56–65% of the weight of the lyophilized LPS preparation.

Table 1. Amino components of lipid A preparations from *P. fluorescens* (% dry weight)

Component	IMB 2108 (biovar II)	IMB 2111 (biovar IV)
Glucosamine	6.80	8.20
Phosphoethanolamine	2.60	3.10
Ethanolamine	0.50	0.65
Aspartic acid	0.71	0.9
Serine	0.43	0.48
Glutamic acid	0.39	0.41
Glycine	0.65	0.83
Alanine	0.64	0.81
Leucine	0.32	0.39
Histidine	0.31	0.35
Lysine	0.29	0.32

Table 2. Fatty acid composition of lipid A from *P. fluorescens*

Peak number, fatty acid (% of the sum total of fatty acids)	*P. fluorescens*	
	IMB 2108 (biovar I)	IMB 2111 (biovar IV)
1. 3-OH-$C_{10:0}$	17.3	6.2
2. $C_{12:0}$	12.3	12.7
3. 2-OH-$C_{12:0}$	18.0	14.0
4. 3-OH-$C_{12:0}$	20.3	20.5
5. $C_{16:1}$	8.3	7.1
6. $C_{16:0}$	16.8	20.2
7. $C_{18:1}$	6.4	13.5
8. $C_{18:0}$	0.6	4.8

Glucosamine and phosphoethanolamine were identified as major components of the hydrophilic portion of lipid A by ion-exchange chromatography (Table 1). Ethanolamine and amino acids (frequently encountered in microbial membrane proteins) were present in minor amounts (in total, not exceeding 4% of the weight of the lipid component). Lipid A is the most evolutionarily conserved portion of the LPS molecules. In all gram-negative bacteria, it is represented by a hydrophilic 1,4-bisphosphorylated β-1,6-diglucosamine backbone, to which a number of fatty acids (mostly hydroxylated) are covalently attached. As expected, quantitative ratios of the major components of the hydrophilic backbone of lipid A in the strains under study were the same as in their counterparts in biovars I and II of *P. fluorescens* (described in our prior publications [5–7]).

Gas–liquid chromatography and gas chromatography–mass spectrometry demonstrated that lipid A contained the same fatty acids (Table 2) that were previously identified in other representatives of *P. fluorescens* [5–7]. The increased content of hexadecanoic

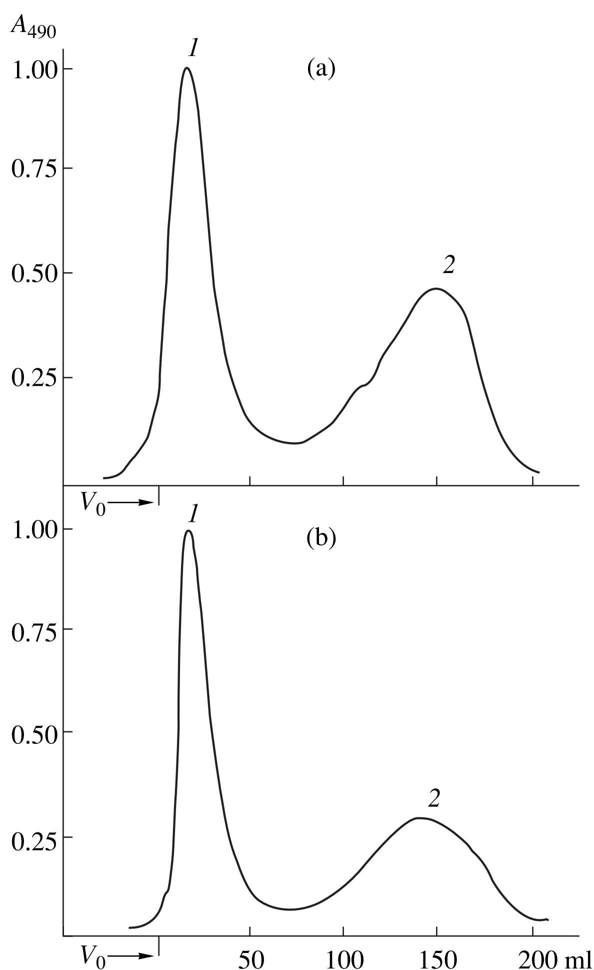

Fig. 1. Elution profile of Sephadex G-50 gel filtration of the carbohydrate moiety of the *P. fluorescens* LPS: (a) IMB 2108 (biovar II), (b) IMB 2111 (biovar IV), (*1*) fraction of O-specific polysaccharide, (*2*) fraction of core oligosaccharide, and (V_0) free volume.

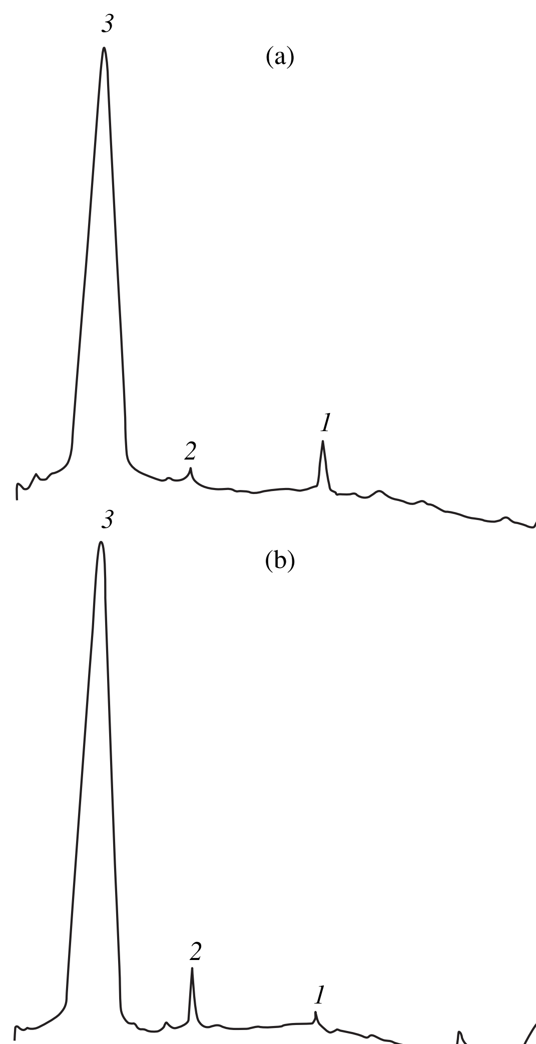

Fig. 2. Separation of the components of acidic hydrolysate (3 N HCl, 4 h) in the O-specific polysaccharides of the *P. fluorescens* strain LPSs by anion-exchange chromatography (BTA 2118 resin; discontinuous gradient of potassium-borate buffers): (a) IMB 2108, (b) IMB 2111, (*1*) mannose, (*2*) galactose, and (*3*) glucose.

acid in the lipid A of the strains under study (16.8 and 20.2%) can be viewed as their characteristic feature. The strains differed with respect to the amount of 3-hydroxydecanoic acid present (17.3 and 6.2%). The strains showed virtually no differences in the amount of the other predominant fatty acids (2-hydro-xydodecanoic, 3-hydroxydodecanoic, dodecanoic, and hexadecanoic), being similar to type strain *P. fluo-rescens* IMB 4125 [6]. Thus, the structures of the lipid A in the LPSs under study were typical of *P. fluorescens* and did not indicate that the strains belonged to distinct chemotypes.

Water-soluble products from mild acidic hydrolysis of the LPS preparations (representing the carbohydrate moiety of the LPS macromolecule) were subjected to Sephadex G-50 chromatography (Fig. 1). The presence of O-specific polysaccharide (peak *1*) and fractions of the core oligosaccharide (peak *2*) indicated that the original preparations were mixtures of S- and R-form LPSs, present at a ratio of 1 : 2, respectively (approximate value). As established previously [5–7], such macromolecular organization of the LPS is characteristic of other strains of *P. fluorescens* and may be a general trait of this species.

When subjected to Sephadex G-50 gel filtration, the preparations of the core oligosaccharide were eluted as a single peak, and their mobility corresponded to that of low-molecular-weight fractions (Fig. 1). The strains under study exhibited certain differences with respect to the composition of this fraction. The preparations contained components (Table 3) that had previously been identified in the core of other strains of *P. fluorescens* [5–7], particularly, rhamnose and alanine. Although these compounds are found in the core oligosaccharides of all the strains of this species studied by us thus far, recent evidence indicates that rhamnose may not necessarily be present, as is the case for type

strain *P. fluorescens* ATCC 49271 [11]. The core portions of the LPSs of the strains under study exhibited certain differences in relation to the amount of their components. The core of the LPS of IMB 2111 lacked arabinose and glucosamine. Heptose was present in trace amounts in the core preparations from both strains. This presence was also noted in other *P. fluorescens* strains [5, 6] and may be a species-specific trait. Thus, our results and other published data indicate that the core portion of the LPS macromolecule in representatives of *P. fluorescens* is heterogenic.

During gel filtration, the carbohydrate fraction (O-specific polysaccharide chains) was eluted with free volume in both the LPSs under study (as well as in the LPSs of other strains of the species that we had previously studied), which indicated that their molecular weights were similar. Rechromatography of the preparations obtained (semipreparative HPLC) showed that the polysaccharides were eluted at a rate similar to that of a 9000 Da standard. Liquid chromatography of the acidic hydrolysates of O-specific polysaccharides identified glucose (Glc) complexed with borate in both preparations. Taking into account the HPLC data, the degree of polymerization of O-specific polysaccharides corresponds to 50 glucose residues. The components of the core portion were revealed in minor amounts. The chromatograms of the polysaccharides of IMB 2108 and 2111 differed only in their minor components (Fig. 2). The data obtained indicate, therefore, that both O-specific polysaccharides are built from glucose residues. The ^{13}C-NMR spectra of the two O-specific polysaccharides, which were recorded under identical conditions, were typical of regular polymers (Fig. 3) and showed little, if any, difference. Each spectrum contained two sets of signals (major and minor). According to the database of NMR signals, the first set (101.7, 79.2, 75.1, 73.4, 73.1, and 62.5 ppm) can be attributed to a 1 ⟶ 4-linked α-glucose homopolymer. The minor set of resonance signals (101.7, 74.8, 74.5, 73.6, 73.2, and 71.4 ppm) can possibly be attributed to an admixture of another homopolymer (such as the carbohydrate component of the core oligosaccharide). This assumption is corroborated by the data obtained via ^{1}H-NMR spectroscopy (Fig. 4, minor signals). In a strong field, signals of the singlets corresponding to methyl groups of *N*-acetates were seen in the range 2.1–2.0 ppm, and three doublets corresponding to methyl groups of 6-deoxy sugars were observed at 1.2–1.1 ppm. The major set of the ^{1}H-NMR spectrum included signals at 5.4 and 5.0 ppm (broadened singlets without a visible multiplet structure) corresponding to anomeric protons, a group of multiplets in the range 4.0–3.6 ppm, and a triplet at 3.4 ppm corresponding to the protons of 2–6 sugar rings. All signals most probably pertain to a glucose ring with an α configuration. Comparison of the chemical shifts occurring in the ^{1}H- and ^{13}C- NMR spectra of the polysaccharides under study with data obtained from signal assignment calculations

Table 3. Composition of the *P. fluorescens* core oligosaccharide

Component (% of total content)	*P. fluorescens*	
	IMB 2108	IMB 2111
Rhamnose	4.3	3.8
Arabinose	5.3	–
Galactose	6.9	19.2
Glucose	39.9	57.7
Heptose	traces	traces
Mannose	41.7	17.3
Component (% of the weight of the dry preparation)		
Alanine	3.1	2.8
Glucosamine	6.4	traces
Galactosamine	traces	0.7
Phosphoethanolamine	0.3	0.6
2-Keto-3-deoxyoctulosonic acid	6.1	2.7
Phosphorus	3.8	5.2

(^{13}C-NMR spectra) and gas–liquid chromatography of the hydrolysates of the preparations of O-specific polysaccharides (following derivatization into (*R*)-2-octyl-glycosides in order to determine the absolute configuration of glucose residues, as described in [11]) demonstrated that the O-specific polysaccharide of *P. fluorescens* IMB 2108 (biovar II) and IMB 2111 (biovar IV) is a homogeneous polymer built from *D*-glucose residues linked together via α-(1 ⟶ 4)glycoside bonds.

It is well known that glucose polymers are nonspecific polysaccharides [12]. They are produced by diverse groups of organisms (plants, algae, fungi, yeasts, and gram-positive or gram-negative bacteria [12–20]) and may be secreted by cells into a medium. An example of the latter case is provided by the extracellular C6-branched β-(1 ⟶ 3)-*D*-glucan, produced by fungi of the genus *Pleurotus* [15]. Glucans have been identified as structural ingredients of the cell wall [16, 17]. They are most frequently found in the periplasmic space [18–20]. Periplasmic glucans are cyclic and have low molecular weights (6–40 glucose residues). A unique periplasmic glucan has been identified in a representative of the genus *Pseudomonas* (*P. syringae* pv. *syringae*). This C6-branched β-(1 ⟶ 2)-*D*-glucan is not cyclic [19], contains 6–13 glucose residues, and has a very low molecular weight. Among the glucans described thus far, those containing α-glycoside bonds are rare. As a rule, they are appear as C6-branched β-(1 ⟶ 3)- or β-(1 ⟶ 2)-glucans.

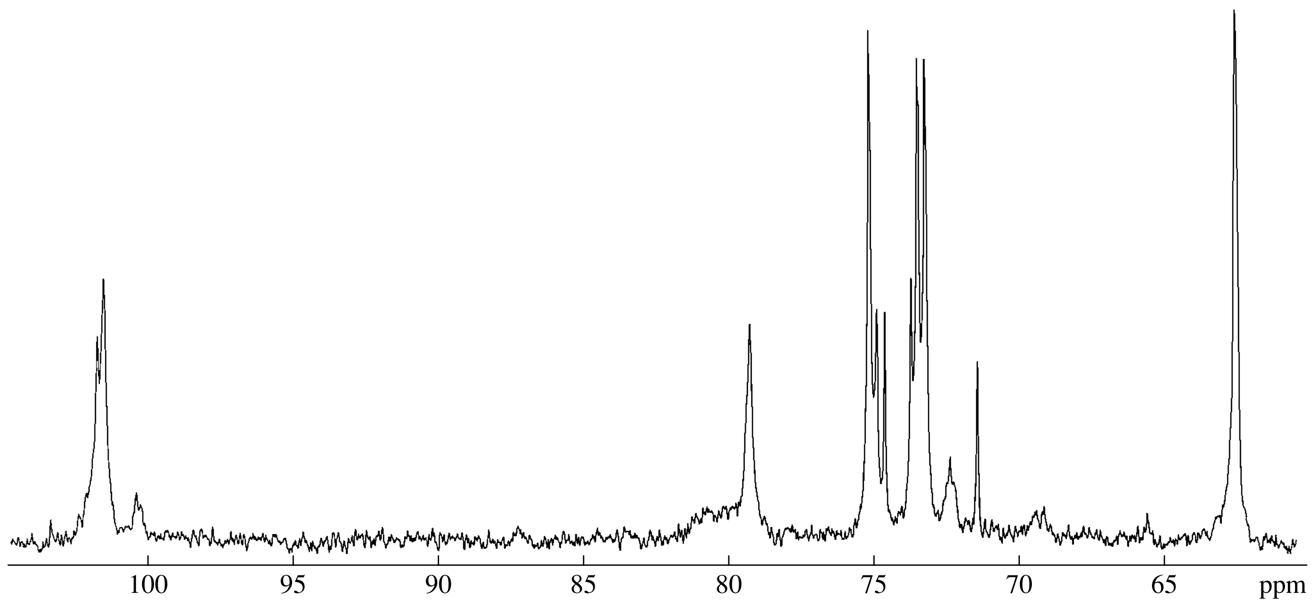

Fig. 3. ^{13}C-NMR spectrum of the O-specific polysaccharide from *P. fluorescens* IMB 2108 (biovar II).

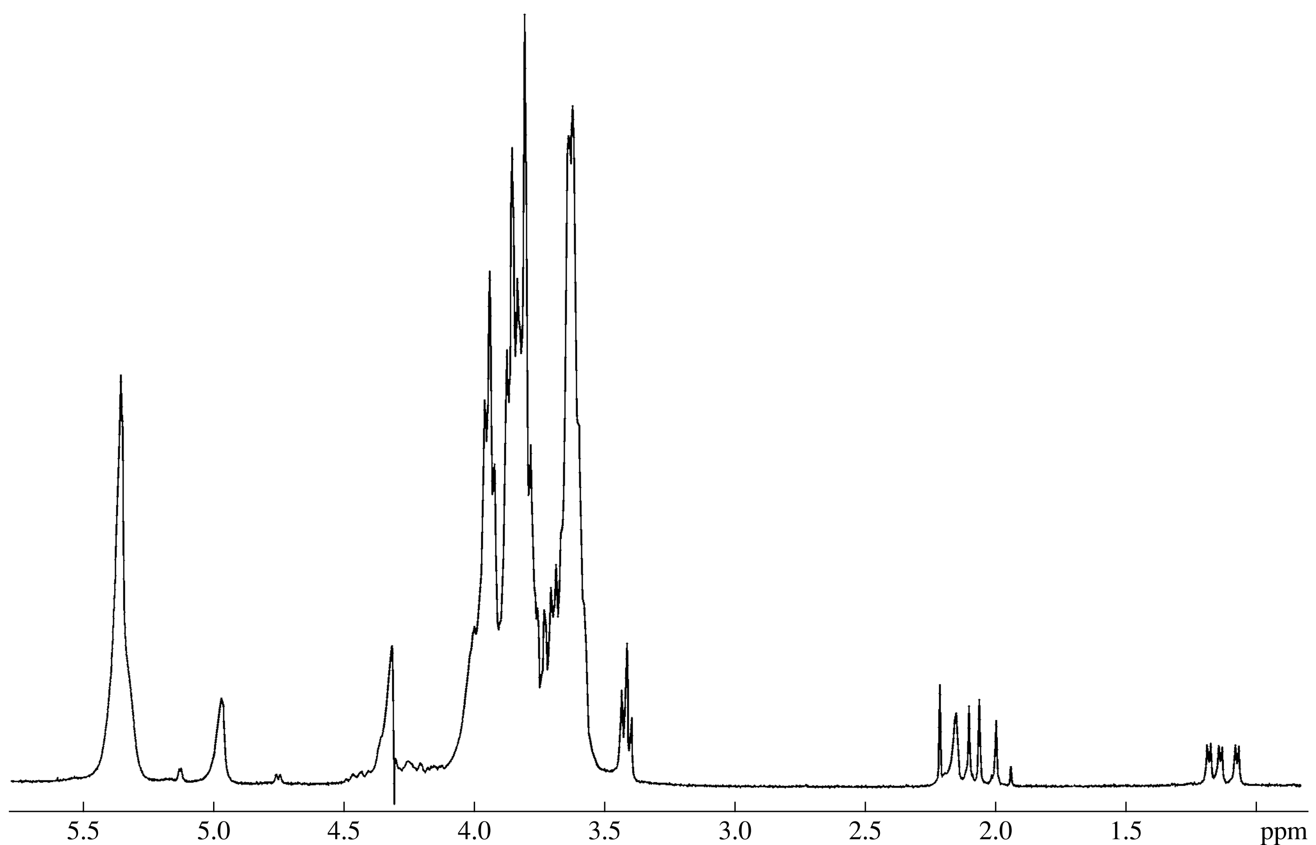

Fig. 4. ^{1}H-NMR spectrum of the O-specific polysaccharide from *P. fluorescens* IMB 2108 (biovar II).

α-(1 ⟶ 4)-linked *D*-glucose residues have been identified in small amounts only in fungi of the genus *Pleurotus* [15]. Periplasmic glucans are known to play an important role in maintaining the osmotic pressure of the periplasm at certain levels. Cell-associated β-glucans may affect the virulence of gram-negative patho-

gens by facilitating tissue colonization and increasing the survival rate within the host [21].

The presence of a glucan in the LPS fractions extracted with hot aqueous phenol from the cells of the *P. fluorescens* strains under study could be the result of coextraction of periplasmic glucans (which are identified in representatives of the genus *Pseudomonas*, as was noted above [19]). These glucans, however, are β-1,2-linked low-molecular-weight polymers. During gel filtration, they are not eluted with free volume. Our preparations were obtained using the classic technique of LPS extraction. Had low-molecular-weight glucans been isolated from microbial cells with hot 45% aqueous phenol, purification by repeated ultracentrifugation (105000 *g* for 3 × 4 h) would have evenly distributed them in the supernatant. It is the supernatant that contained the α(1 ⟶ 4)-*D*-glucan of *Yersinia pseudotuberculosis* (serovar VI) [22]. Also identified in *P. fluorescens* is a branched α-(1 ⟶ 6)-*D*-glucan, which is clearly distinct from the glucan reported in this study. Moreover, in our case, glucans were obtained following acidic hydrolysis (under mild conditions) of the original purified LPS preparation and only after separation of the water-insoluble lipid A fraction and Sephadex G-50 gel filtration of the carbohydrate residue. According to the available data, the glucan described here is unique in both its prevalence and location within the microbial cells. The *P. fluorescens* α-(1 ⟶ 4)-*D*-glucan, identified for the first time in *P. fluorescens* strains, serves as the O-chain of their LPS macromolecules; therefore, it may exhibit functional differences from other glucans. These as yet unknown functions performed by the glucans in microbial cells may well account for the presence of an additional set of signals in the NMR spectra of the LPS preparations of both of the strains under study. Based on preliminary evidence, this set may correspond to rhamnan.

Thus, the LPSs of *P. fluorescens* IMB 2108 (biovar II) and IMB 2111 (biovar IV) are constructed according to the principle common to all gram-negative bacteria, which assumes the existence of three distinct structural portions within the LPS macromolecule (lipid A, core oligosaccharide, and O-specific polysaccharide). The original bulk of the LPS preparations contained both R- and S-type LPS molecules, similarly to LPSs of previously studied *P. fluorescens* strains.

The lipid A portions in the LPSs of the strains under study were found to contain hydrophilic components (glucosamine and phosphoethanolamine) and fatty acids (3-hydroxydecanoic, 2-hydroxydodecanoic, 3-hydroxydodecanoic, and dodecanoic) characteristic of the lipid A of typical representatives of the genus *Pseudomonas*. The lipid A portions in the LPSs of the strains under study did not differ from those of other *P. fluorescens* strains with respect to the structure of the hydrophilic part. Thus, the structural organization of lipid A in the LPSs under study was the same as in the LPSs of the other biovars of *P. fluorescens*.

The core oligosaccharides of the LPSs under study exhibited certain differences from their previously studied counterparts. The core portions of the two LPSs also differed from each other in the quantity of components present. Our results and other published data indicate that this portion of the LPS macromolecule is heterogeneous in representatives of *P. fluorescens*.

The structure of the O-specific polysaccharide of the LPSs of *P. fluorescens* IMB 2108 and IMB 2111 is unique in that the polymer chain appears as a homopolymer of *D*-glucose residues linked together via α-(1 ⟶ 4)-glycoside bonds, which is in contrast to what was found in the LPSs of other strains of this bacterial species. The identity of the structure of O-chains of the LPSs under study was also confirmed in immunological cross-reactions, which indicated that the only possible immunodominant fragment was related to *D*-glucose residues. The lack of serological interrelations of the strains under study to those representing other *P. fluorescens* biovars correlates with the known strain heterogeneity of this taxon (hence, the division of its strains into biovars and biovar heterogeneity). At the same time, the serological similarity of the strains under study may be taken as an indication of their affiliation with the same biovar of the species *P. fluorescens*.

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