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EXPERIMENTAL ARTICLES

Peculiarities of the Structure of the *Pseudomonas fluorescens* IMV 247 (Biovar II) Lipopolysaccharide

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Abstract—The results of the study of the *Pseudomonas fluorescens* IMV 247 (biovar II) lipopolysaccharide (LPS) isolated from the dry bacterial mass by Westphal's method and purified by repeated ultracentrifugation are presented. The macromolecular organization of the LPS is characterized by the presence of S and R forms of LPS molecules in a 1 : 1 ratio. The structural components of the LPS molecule—lipid A, the core oligosaccharide, and the *O*-specific polysaccharide—were isolated and characterized. 3-Hydroxydecanoic, 2-hydroxydodecanoic, 3-hydroxydodecanoic, and dodecanoic acids proved to be the main lipid A fatty acids. Glucosamine, phosphoethanolamine, and phosphorus were identified as the components of the lipid A hydrophilic portion. Glucose, galactose, arabinose, rhamnose, glucosamine, galactosamine alanine, phosphoethanolamine, phosphorus, and 2-keto-3-deoxyoctulonate (KDO) were revealed in the heterogeneous fraction of the core oligosaccharide. The *O*-specific polysaccharide chain was composed of repeating tetrasaccharide units consisting of L-rhamnose (L-Rha), 3,6-dideoxy-3-[(*S*)-3-hydroxybutyramido]-D-glucose (D-Qui3NHb), 2-acetamido-2,4,6-trideoxy-4[(*S*)-3-hydroxybutyramido]-D-glucose (D-QuiNAc4NHb), and 2-acetamido-2-deoxy-D-galacturonic acid (D-GalNAcA) residues. A peculiarity of the *O*-specific polysaccharide was that it released, upon partial acid hydrolysis, the nonreducing disaccharide GalNAcA ⟶ QuiNAc4NHb with a 3-hydroxybutyryl group glycosylated intramolecularly with a QuiN4N residue. Double immunodiffusion in agar and lipopolysaccharide precipitation reactions revealed no serological interrelationship between the strain studied and the *P. fluorescens* strains studied earlier.

Key words: lipopolysaccharide, *Pseudomonas fluorescens*, lipid A, core polysaccharide

Because of their heterogeneity, *P. fluorescens* strains are divided into five biovars according to phenotypic and genotypic characteristics [1]. The strains of the bacteria of biovar II (biotype B according to Stanier [2]) can be isolated from different sources: water, soils, foodstuffs, and the plant rhizosphere. This taxon, like the other biovars of the species, is heterogeneous. Numerical analysis singled out three phenogroups [3] whose taxonomic rank has not yet been determined. No serological classification of these bacteria has been elaborated. There are data [4] on the similarity between biovar II strains and bacteria of the species *P. aurantiaca* included in section V of the genus *Pseudomonas* (this section comprises species whose taxonomic position has not been definitively determined). The DNA homology between strains of the *P. fluorescens* biovar II and *P. aurantiaca* can be as high as 90–100%, which, taking into account other characteristics, allows the bacteria of the species *P. fluorescens* biovar II to be considered pigment-free variants of *P. aurantiaca*.

Earlier [5–7], we studied the LPS of strain IMV 1602, the most typical representative of the *P. fluorescens* biovar II. A similarity with the *P. aurantiaca* LPS in the lipid A fatty acid composition was revealed, suggesting phylogenetic relatedness; however, differences were revealed in the structure of the core portion and the *O*-specific polysaccharide chain. By a number of characteristics, the LPS of strain IMV 1602 was similar to the LPS of the type strain *P. fluorescens* IMV 4125. However, to use the data on the LPS structure as a chemotaxonomic criterion, it is necessary to study the LPS of a larger number of strains.

The aim of this work was to study the LPS of strain *P. fluorescens* IMV 247 belonging to biovar II; to isolate and study certain structural and functional parts of the LPS molecule—lipid A, the core oligosaccharide, and the *O*-specific polysaccharide chain—and to determine the degree of similarity with the LPS of other *P. fluorescens* biovar II strains studied earlier.

MATERIALS AND METHODS

Strain *P. fluorescens* IMV 247 (biovar II) from the collection of microorganisms of the Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, was kindly provided by E.A. Kiprianova.

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The bacteria were grown at 28°C on nutrient agar for 28 h. The bacterial mass was washed off with a saline solution, precipitated by centrifugation, washed with saline, and dried with acetone and diethyl ether. *O*-sera were obtained by immunizing rats with suspensions of bacterial cells (4×10^9 /ml) killed by heating at 100°C for 2.5 h. Serological studies were conducted using the techniques described earlier [5]. LPSs from the dry bacterial mass were isolated by phenol–water extraction [8] at 65°C and purified by triple ultracentrifugation at 105000 g. The precipitate was dried lyophilically. Electrophoresis of LPS samples in polyacrylamide gel with SDS was carried out at 0.04 A for 2 h. The stacking gel contained 3% acrylamide; the separating gel contained 10% acrylamide. Before being applied to the wells, LPS samples were heated at 100°C for 10 min. The gel was stained with a silver nitrate-based stain as described by Tsai [17]. To isolate lipid A, the core oligosaccharide, and *O*-specific polysaccharide fractions, the LPS sample was subjected to mild acidic hydrolysis with 1% acetic acid (1.5 h, 100°C). The water-insoluble lipid A fraction was separated by centrifugation. The fractions corresponding to the core oligosaccharide and the *O*-specific polysaccharide chain were isolated by gel filtration of the LPS carbohydrate portion on a column with Sephadex G-50 [7]. High performance liquid chromatography (HPLC) of *O*-specific polysaccharide was carried out using the Gold System device (Beckman, USA) on a TSK SW 4000 (7.5×300 mm) column with H_2O as an eluent and spectrophotometric detection at 200 nm; HPLC of the core oligosaccharide was carried out on a μSpherogel-Carbohydrate (300×6.5 mm) column at 80°C with H_2O as an eluent and a differential refractometric detector (Knauer, Germany). Lipid A fatty acids were methylated in sealed ampoules with 1.5 M HCl in methanol (100°C, 3 h); methyl esters were analyzed using a Chrom-5 gas chromatograph (Czechia) with a flame ionization detector and helium as a carrier gas on a column ($1.2 \text{ m} \times 3$ mm) with 5% SE-30 on Chromaton N-AW-DMCS, as well as on a $2 \text{ m} \times 3$ mm column with 5% DEGS-PS on Supelcoport (100–120 mesh). Trifluoroacetylation of hydroxy acid methyl esters was carried out as described earlier [6]. The derivatives obtained were analyzed on a gas chromatograph under the above conditions. 2-Keto-3-deoxyoctulonate (KDO) was identified by the reaction with thiobarbituric acid [9]. Phosphorus was determined as described earlier [7]. The total carbohydrate content in LPS samples was determined by the reaction with phenol and sulfuric acid [18]. Neutral sugars in the hydrolysates (2N HCl, 4 h, 100°C) of LPS carbohydrate constituent samples were analyzed by ion exchange chromatography on a column with the BTA 2118 anion exchange resin (Biotronik, Germany) in the system of the stepped gradient of potassium–boracic buffers [10], as well as by GLC of polyol acetates as described earlier [5]. ^1H and ^{13}C NMR spectra were recorded in D_2O at 50 or 60°C using Brucker AM-300, AM-500, and DRX-500 devices. Acetone (δ_H 2.23, δ_c 31.45) was used as the internal standard. Spectrophotometric analysis of LPS was carried out using a DU-8B spectrophotometer (Beckman, USA).

RESULTS AND DISCUSSION

The LPS of *P. fluorescens* IMV 247 (biovar II), extracted from the dry bacterial mass with hot phenol and purified by ultracentrifugation, exhibited an absorption band at 200–220 nm and a minor band at 245–280 nm due to an admixture of proteins and nucleic acids. The LPS contained 42% carbohydrates (quantified by the reaction with phenol and sulfuric acid) and 4% phosphorus (quantified by the Fiske–Subbarow method). The LPS yield was 2.9% of the dry weight of the bacterial mass acetone powder.

The sample exhibited serological activity with a homologous *O*-serum in the reactions of double immunodiffusion in agar (two lines) and precipitation (titer, 1 : 500 000) but not in cross reactions with *O*-sera to the neotype *P. fluorescens* strain, strains representing other biovars of the species (including the biovar II strain IMV 1602), or strain *P. aurantiaca* IMV 31.

To obtain preparations of the carbohydrate portion and lipid A fraction, the initial LPS was treated with 1% acetic acid. The water-insoluble lipid A fraction comprised 40% of the weight of the lyophilized LPS preparation.

Glucosamine and phosphoethanolamine were identified as the main components of the lipid A hydrophilic portion by ion exchange chromatography (Table 1); ethanolamine and a series of amino acids common to microbial proteins were present in minor quantities (Fig. 1). The quantitative ratio of the main components was the same as in lipid A of another biovar II strain and biovar I strains studied earlier [11–13].

On the basis of data from GLC and chromatography–mass spectrometry (identification characteristics are given in [13]), we identified in lipid A of the LPS studied fatty acids (Fig. 2) found in the other *P. fluorescens* representatives, in particular, in the lipid A of strain IMV 1602, also representing biovar II [15]. As can be seen from Table 2, the essential difference between the lipid A fatty acid spectrum of the strain studied and that of strain 1602 is the 2-OH$C_{12:0}$ to $C_{12:0}$ ratio (1 : 1 and 1 : 12, respectively), as well as a larger total amount (67.8%) of hydroxy acids (3-OH-$C_{10:0}$, 2-OH$C_{12:0}$, 3-OH-$C_{12:0}$) in the strain studied. A relatively low content of saturated and unsaturated fatty acids with 16 and 18 carbon atoms seems to be a characteristic feature of the lipid A of the two strains compared. It should be noted that the lipid A fatty acid composition of the strain studied is very similar to that of the LPS of *P. aurantiaca* IMV 31 studied earlier [6].

The elution profile of the carbohydrate constituent of strain IMV 247 LPS (Fig. 3, gel filtration on a column with Sephadex G-50) suggests that the initial prep-

Table 1. Components of the hydrophilic portion of lipid A in *P. fluorescens* (biovar II)

P. fluorescens (biovar II), IMV	Glucosamine		Phosphoethanolamine		Phosphorus	Amino acids
	%	mmol	%	mmol	%	%
247	5.10	0.26	1.81	0.12	3.80	1.00
1602	4.90	0.25	1.60	0.10	2.90	2.40

Table 2. Fatty acid composition of lipid A in *P. fluorescens* (biovar II) (% of total fatty acids)

Peak number, fatty acid		*P. fluorescens* IMV	
		247	1602 [15]
1	3-OH-$C_{10:0}$	27.0	15.6
2	$C_{12:0}$	12.7	42.5
3	2-OH-$C_{12:0}$	13.8	3.4
4	3-OH-$C_{12:0}$	27.8	22.7
5	$C_{16:1}$	2.9	6.1
6	$C_{16:0}$	4.1	7.5
7	$C_{18:1}$	1.6	2.4
8	$C_{18:0}$	1.4	0.0

Table 3. Composition of the core oligosaccharide of *P. fluorescens* (biovar II)

Component	*P. fluorescens* IMV	
	247	1602 [7]
(% of the total content)		
Rhamnose	32.6	21.6
Arabinose	1.5	14.8
Galactose	3.0	12.3
Glucose	26.3	33.3
Heptose	Traces	Traces
Mannose	Traces	18.5
(% of the dry weight)		
Alanine	7.0	1.1
Glucosamine	6.5	Traces
Galactosamine	3.9	0.3
Phosphoethanolamine	0.8	3.7
2-Keto-3-deoxyoctulonate	5.9	7.5
Phosphorus	4.8	2.75

aration was a 1 : 1 mixture of the S and R forms of the LPS molecules. As was established earlier [15], the same macromolecular LPS organization is typical of another *P. fluorescens* strain (IMV 1602) assigned to biovar II, as well as of the LPS of biovar I strains [11–13]. Thus, taking into account the identical conditions of bacterial growth and LPS isolation, it may be concluded that biovar II strains are also characterized by a mixed macromolecular LPS organization (if the possibility of cell dissociation in the process of large-scale cultivation of bacteria on solid nutrient medium is to be ruled out).

Analysis of the core oligosaccharide fraction by HPLC revealed three components with different chromatographic mobilities and a peak area ratio of 1 : 2 : 1, which most likely represent core oligosaccharide fragments with a different degree of completion of the process of biosynthesis. A heterogeneous preparation of the core oligosaccharide contained components (Table 3) discovered earlier in this portion of the LPS macromolecules of other *P. fluorescens* strains [7, 11–13]. The heptose component of the internal core region was revealed by HPLC (as polyol acetate) at the trace level, which is also a structural peculiarity of the core portion of the LPS molecules of the *P. fluorescens* strains studied earlier [13]. In contrast to the LPS core portion of the previously studied strain IMV 1602 [7], mannose was revealed only in trace amounts, while the amount of glucosamine was 6.5% (only traces in strain 1602). The core sample studied contained rhamnose and alanine, also found in the LPS core of all *P. fluorescens* strains studied earlier. The degree of phosphorylation of the core portion of the LPS studied in the present work was higher than in the reference sample of strain IMV 1602. Since the amount of phosphoethanolamine in the LPS studied was significantly lower than in the LPS core of strain IMV 1602, it can be suggested that, in strain IMV 247, other components of the internal core portion are phosphorylated, as in the LPS core of *P. fluorescens* ATCC 49271 [18], where the heptose residue is phosphorylated and phosphoethanolamine is absent. The core oligosaccharide of strain IMV 247 also differs from that of *P. fluorescens* ATCC 49271 in other components: the structural level and where fucosamine and quinovosamine are present in the latter strain, while rhamnose is absent. The discovery of rhamnose in the LPS core of *P. fluorescens* IMV 247, as well as in the core of the LPS studied earlier, might be accounted for by minor quantities of the fragments of *O*-chains, where it is a structural component, but this is inconsistent with the detection of rhamnose in the core of the LPS whose *O*-chains do not contain this residue [11, 13]. The amount of KDO, a core region component that is specific for the LPS of gram-negative bacteria, was 6.9 and 7.5% in the biovar II strains compared; in biovar I strains, this value does not exceed 1%, which suggests structural differences in the internal portion of the LPS core region of biovar I and biovar II strains. Thus, the heterogeneity in the composition of

the LPS core revealed in bacteria representing different biovars of the species *P. fluorescens* agrees with their well-known heterogeneity with respect to phenotypic and genotypic characteristics [1].

The preparation of the *O*-specific polysaccharide fraction of the strain IMV 247 LPS isolated by gel filtration emerged, when analyzed by HPLC, in the region of the 5×10^4-Da molecular mass standard as a symmetrical peak, with a retention of the rear peak front indicative of the presence of polysaccharide chains with a lesser number of repeating units. This heterogeneity and the average molecular mass value were also confirmed by electrophoresis in polyacrylamide gel with SDS. 3-Amino-3,6-dideoxyglucose (Qui3N) was identified in the polysaccharide acidic hydrolysate by using an amino acid analyzer; galactosamine and the amino component eluted in the emergence zone of bacillosamine were identified in minor quantities (Fig. 4). Analysis of the components of the same hydrolysate in the form of boracic complexes by liquid chromatography, as well as in the form of polyol acetates by GLC, revealed rhamnose. These data seem to suggest that the *O*-specific polysaccharide is made up of the rhamnose and quinovosamine residues (the minor components revealed could be accounted for by the presence of the LPS core portion in the polysaccharide due to the mild conditions of the hydrolysis with acetic acid used to cleave the acid-labile bond between lipid A and the carbohydrate portion [7]). However, it follows from the NMR spectroscopy data that the polysaccharide has a more complex structure and includes other structural components and substituents. Thus, the polysaccharide ^{13}C NMR spectrum (Fig. 5, Table 4) exhibited the signals of four anomeric carbon atoms in the 96.1–105.8 ppm range, four carbon atoms bonded with nitrogen in the 50.6–57.9 ppm range, three CH_3-C groups of 6-deoxyhexoses (C-6) in the 17.7–18.1 ppm range, one COOH group of uronic acid at 174.0 ppm, and 12 other carbon signals in the 68.5–81.1 ppm range. Moreover, the signals of two *N*-acetyl and two *N*-(3-hydroxybutyryl) groups were present (CH_3 and CO of both groups at 23.7–23.9 and 174.9–175.6 ppm, respectively; CH-OH and CH_2 of the 3-hydroxybuytryl groups at 66.0 and 46.1–46.3 ppm, respectively). These data testify to the fact that the polysaccharide has a regular linear structure and is made up of repeating tetrasaccharide units.

The ^1H NMR-spectrum of the *O*-specific polysaccharide (Table 5) included the signals of four anomeric protons at 4.66 ($J_{1,2}$7.5 Hz), 5.12 ($J_{1,2}$3.5 Hz), 5.39 ($J_{1,2} < 2$ Hz), and 5.57 ppm ($J_{1,2}$3.5 Hz), five CH_3-C groups of three 6-deoxyhexoses and two *N*-(3-hydroxybutyryl) groups at 1.10–1.19 ppm, two CH_2 groups at 2.21, 2.27, 2.34, and 2.42 ppm, as well as two N-acetyl groups at 1.93 and 1.96 ppm.

Further analysis of the NMR spectroscopy data and data from different variants of correlation spectroscopy showed that the repeating unit of the *P. fluorescens*

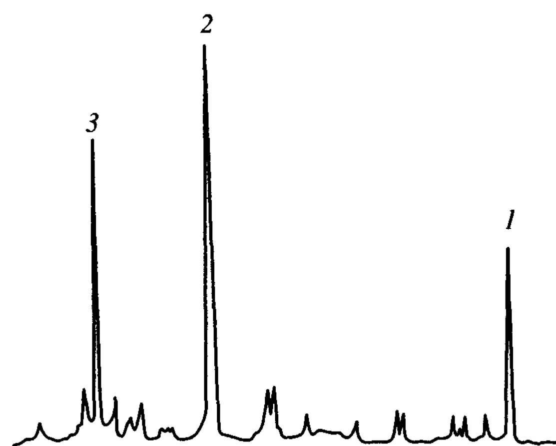


Fig. 1. Chromatogram of lipid A hydrolysate (4 N HCl, 6 h) of *P. fluorescens* IMV 247 (biovar II) on an amino acid analyzer in the system of sodium–citrate buffers. (*1*) Phosphoethanolamine; (*2*) glucosamine; (*3*) ammonia. Minor peaks correspond to amino acids.

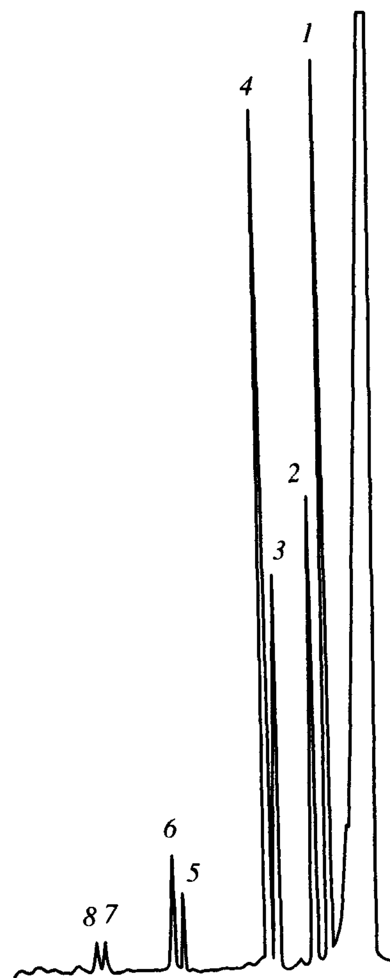


Fig. 2. GLC profile of fatty acid methyl esters of *P. fluorescens* IMV 247 (biovar II) lipid A: (*1*) 3-OH-$C_{10:0}$; (*2*) $C_{12:0}$; (*3*) 2-OH-$C_{12:0}$; (*4*) 3-OH-$C_{12:0}$; (*5*) $C_{16:1}$; (*6*) $C_{16:0}$; (*7*) $C_{18:1}$, and (*8*) $C_{18:0}$.

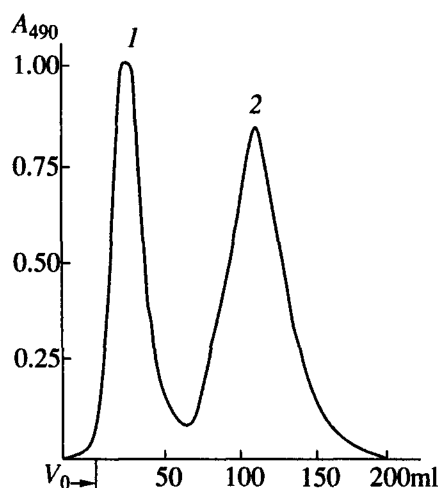


Fig. 3. Gel filtration of the LPS carbohydrate portion of *P. fluorescens* IMV 247 (biovar II) on a column with Sephadex G-50: (*1*) *O*-specific polysaccharide fraction; (*2*) the core oligosaccharide fraction; V_0 is the free volume.

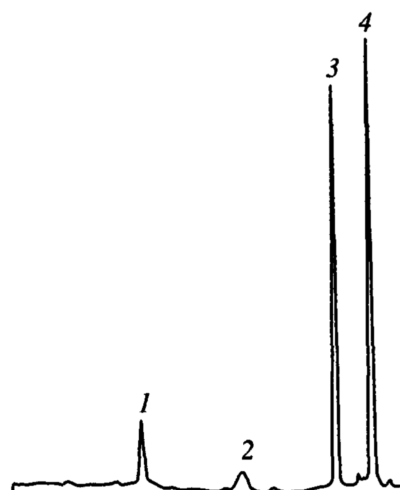
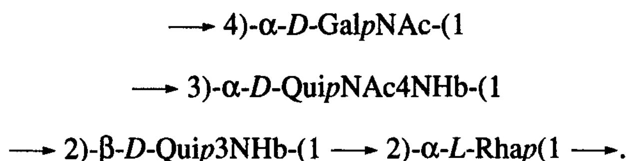


Fig. 4. Chromatogram of the *O*-specific polysaccharide hydrolysate (3 N HCl, 4 h) of *P. fluorescens* IMV 247 (biovar II) on an amino acid analyzer: (*1*) unidentified component; (*2*) glucosamine; (*3*) quinovosamine; and (*4*) ammonia.

IMV 247 *O*-specific polysaccharide has the following structure:



→ 4)-α-*D*-Gal*p*NAc-(1

→ 3)-α-*D*-Qui*p*NAc4NHb-(1

→ 2)-β-*D*-Qui*p*3NHb-(1 → 2)-α-*L*-Rha*p*(1 →.

The detailed data concerning the identification of the structure of the repeating polysaccharide unit were published earlier [14].

The specific feature of the *O*-specific polysaccharide of *P. fluorescens* IMV 247 (biovar II) was that upon partial acidic hydrolysis, it released a disaccharide that contained 2-amino-2-deoxygalacturonic acid (GalNA) and 2,4-diamino-2,4,6-trideoxyglucose (QuiN4N). Obviously, the absence of these monosaccharides in the hydrolysate of the *O*-specific polysaccharide was determined by disaccharide resistance under standard hydrolysis conditions. NMR spectroscopy showed that the disaccharide lacked a free reducing end and was an intramolecular bicyclic glycoside of the 3-hydroxybutyryl group [14].

Thus, the LPS of *P. fluorescens* IMV 247 has a structure typical of all gram-negative bacteria and contains all of the characteristic structural parts, namely, lipid A, the core oligosaccharide, and the *O*-specific polysaccharide chains. The LPS pool contained both R and S forms of LPS molecules; thus, mixed macromolecular LPS organization is characteristic not only of the LPS of *P. fluorescens* biovar I strains but also of the LPS of biovar II strains. Glucosamine, phosphoethanolamine, and phosphorus, as well as fatty acids typical of the lipid A of fluorescent bacteria (3-hydroxydecanoic, 2-hydroxydodecanoic, 3-hydroxydodecanoic, and dodecanoic fatty acids) were found in lipid A. The LPS studied did not differ in the composition of the lipid A hydrophilic portion from the LPS studied earlier, whereas certain differences were revealed in the composition of fatty acids and the degree of phosphorylation relative to strain *P. fluorescens* IMV 1602 (biovar II) studied earlier. Strain IMV 247 was similar to *P. aurantiaca* IMV 31 in the lipid A fatty acid compo-

Table 4. Chemical shifts (δ, ppm) in the ^{13}C NMR spectrum of the *O*-specific polysaccharide of *P. fluorescens* IMV 247 (biovar II)

Monosaccharide							Ac		Hb			
	C-1	C-2	C-3	C-4	C-5	C-6	C-1	C-2	C-1	C-2	C-3	C-4
→4)-α-*D*-Gal*p*NAc-(1→	98.6	50.6	69.4	76.2	72.4	174.0	175.7	23.7				
→3)-α-*D*-Qui*p*NAc4NHb-(1→	96.1	53.5	74.7	57.9	68.5	17.8	175.4	23.7	174.9	46.3	66.0	23.7
→2)-β-*D*-Qui*p*3NHb-(1→	105.8	75.1	56.9	74.7	74.1	17.7			175.6	46.1	66.0	23.7
→2)-α-*L*-Rha*p*(1→	100.9	81.1	70.7	73.7	70.2	18.1						

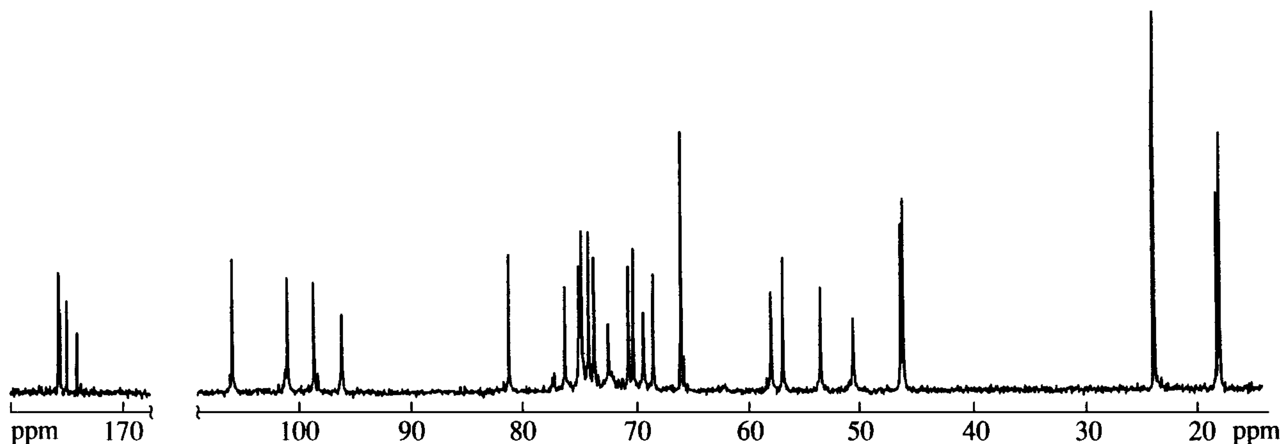


Fig. 5. ^{13}C NMR spectrum of the *O*-specific polysaccharide of *P. fluorescens* IMV 247 (biovar II).

sition and had a relatively low content of saturated and unsaturated fatty acids with 16 and 18 carbon atoms. As for the component composition of the core portion, the LPS studied was very similar to another biovar II representative (IMV 11602), except for the absence of mannose and a greater content of alanine and glucosamine. The possibility of phosphorylation of other structural components of the core portion can be inferred from a lower amount of phosphoethanolamine and an increased amount of phosphorus in the LPS core of the strain studied relative to strain IMV 1602 (biovar II). Taking into account a large amount of KDO relative to biovar I strains, differences in the structure of the internal core portion are possible. The structure of the LPS *O*-specific polysaccharide chains of strain INV 247 is unique not only within *P. fluorescens* but also for all pseudomonas. The occurrence of three amino sugars in the repeating unit of the *O*-specific polysaccharide was discovered only in several bacteria, namely, in the *O*-specific polysaccharides of *P. fluorescens* IMV 1152 [11] and IMV 1433 [13] and *P. aurantiaca* IMV 31 [19]. Worthy of note is also the formation of intramolecular glycoside, discovered earlier only for the N-(3-hydroxybutyryl) derivative of QuiN4N in the disaccharide and trisaccharide of the *O*-specific polysaccharide of *P. aeruginosa* (ATCC 27577) [14].

The absence of a serological relationship with the other biovar II representative and the biovar I strains gives evidence of the heterogeneity of the LPS of the taxon strains studied. The revealed similarity in some components between the LPS studied in this work and the LPS of *P. aurantiaca* IMV 31 correlates with the high DNA homology between *P. fluorescens* and *P. aurantiaca* strains [4]; however, the taxonomic significance of the data obtained cannot be appreciated until

Table 5. Chemical shifts (δ, ppm) and constants of the spin–spin interaction (*J*, Hz) of the ^1H NMR spectrum of the *O*-specific polysaccharide of *P. fluorescens* IMV 247 (biovar II)

Monosaccharide							Ac	Hb			
	H-1	H-2	H-3	H-4	H-5	H-6	H-2	H-2a	H-2b	H-3	H-4
→4)-α-*D*-Gal*p*NAc-(1→	5.12	4.16	3.80	4.26	4.06		1.96				
	$J_{1,2}$ 3.5	$J_{2,3}$ 11	$J_{3,4}$ 3	$J_{4,5}$ <2							
→3)-α-*D*-Qui*p*NAc4NHb-(1→	5.57	3.99	3.72	3.75	3.62	1.10	1.93	2.27	2.21	4.11	1.16
	$J_{1,2}$ 3.5	$J_{2,3}$ 9	$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6}$ 6			$J_{2a,3}$ 8	$J_{2a,2b}$ 15	$J_{2b,3}$ 5	$J_{3,4}$ 6
→2)-β-*D*-Qui*p*3NHb-(1→	4.66	3.38	3.86	3.07	3.39	1.14		2.42	2.34	4.21	1.19
	$J_{1,2}$ 7.5	$J_{2,3}$ 9.5	$J_{3,4}$ 9.5	$J_{4,5}$ 9.5	$J_{5,6}$ 6			$J_{2a,3}$ 8	$J_{2a,2b}$ 15	$J_{2b,3}$ 5	$J_{3,4}$ 6
→2)-α-*L*-Rha*p*(1→	5.39	3.96	3.72	3.20	3.55	1.14					
	$J_{1,2}$ <2	$J_{2,3}$ 3.5	$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6}$ 6						

more strains representing the *P. fluorescens* biovar II have been studied in greater detail.

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