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Characterization of the Lipopolysaccharide and Structure of the O-Specific Polysaccharide of the Bacterium *Pseudomonas syringae* pv. *atrofaciens* IMV 948

G. M. Zdorovenko[1]*, A. S. Shashkov[2], E. L. Zdorovenko[2], N. A. Kocharova[2], L. M. Yakovleva[1], Yu. A. Knirel[2], and K. Rudolph[3]

[1]Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, ul. Zabolotnogo 154, Kiev, 252143 Ukraine; fax: (044) 266-2379; E-mail: zdorov@i.kiev.ua
[2]Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii pr. 47, Moscow, 117913 Russia; fax: (095) 135-5328; E-mail: knirel@ioc.ac.ru
[3]Institut für Pflazenpathologie und Pflanzenschutz, Georg-August-Universität, Grisebachstrasse 6, 37077 Göttingen, Germany; fax: (49) 551-394187; E-mail: krudolp@gwdg.de

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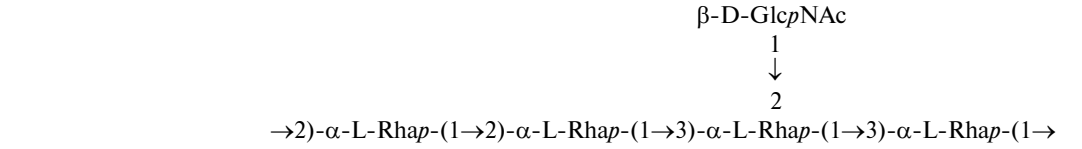
Abstract—Lipopolysaccharide (LPS) was isolated from the phytopathogenic bacterium *Pseudomonas syringae* pv. *atrofaciens* IMV 948 by mild extraction of the microbial cells with saline, and the properties, composition, and structure of the LPS were studied. The LPS showed low toxicity in D-galactosamine-sensitized mice and low biological activity in plants. Structural components of LPS—lipid A, core oligosaccharide, and O-specific polysaccharide (OPS)—were obtained by mild acid degradation and characterized. The lipid A contained fatty acids 3-HO-$C_{10:0}$, $C_{12:0}$, 2-HO-$C_{12:0}$, 3-HO-$C_{12:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$, as well as components of the hydrophilic moiety: GlcN, ethanolamine, phosphate, and phosphoethanolamine. The LPS core contained components typical of pseudomonads: glucose, rhamnose (Rha), L-*glycero*-D-*manno*-heptose, GlcN, GalN, 2-keto-3-deoxy-D-*manno*-octonic acid, alanine, and phosphate. The OPS consisted of L-Rha and D-GlcNAc in the ratio 4 : 1 and was structurally heterogeneous. The main pentasaccharide repeating unit of the OPS has the following structure:



Immunochemical studies showed that *P. syringae* pv. *atrofaciens* IMV 948 is serologically separate from other *P. syringae* strains, including those that have structurally similar OPS.

Key words: *Pseudomonas syringae*, lipopolysaccharide, composition, structure, immunochemistry

The species *Pseudomonas syringae* consolidates a large group of the phytopathogenic bacteria differing in host and serological specificity. Accordingly, they are divided into pathovars [1-4] and serogroups [4-7]. The fine structure of the O-specific polysaccharide (OPS) chain of the lipopolysaccharide (LPS) that is located in the outer membrane of the microbial cell wall defines the immunospecificity of Gram-negative bacteria, including *P. syringae* [8, 9]. LPS plays an important role in the processes of recognition and interaction of the bacteria with the host plant.

LPS from *P. syringae* strains of different pathovars and serotypes are being studied systematically aiming at correlation between their structure, immunospecificity, and host specificity [10, 11]. OPSs of *P. syringae* have been found to possess similar structures and consist of oligosaccharide repeating units that contain three or four L-Rha or/and D-Rha residues in the backbone and, in addition, may have a lateral sugar substituent (D-Rha, D-Fuc, 3-acetamido-3,6-dideoxy-D-galactose or D-

* To whom correspondence should be addressed.

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GlcNAc). Some questions on systematic and immunospecificity of the *P. syringae* were based on the molecular level by using data on the OPS structure. Immunochemical studies using monoclonal antibodies enabled inferring serogroup- and serotype-specific epitopes on the LPS [5, 6, 10-13].

In continuation of the systematic investigations of *P. syringae* strains, we isolated and characterized the whole LPS and studied the OPS structure of *P. syringae* pv. *atrofaciens* IMV 948 belonging to serogroup V according the classification scheme of Pastushenko and Simonovich [5]. Now we report the results of these studies.

MATERIALS AND METHODS

Strain *Pseudomonas syringae* pv. *atrofaciens* IMV 948 from the bacterial collection of the Zabolotny Institute of Microbiology and Virology (Kiev) was grown on potato dextrose agar at 26-28°C for 20-22 h.

The LPS was isolated in a yield of ~10% from crude microbial biomass by extraction with 0.85% saline and purified by repeated ultracentrifugation (3 × 4 h) at 105,000*g*.

The LPS was degraded with 1% acetic acid at 100°C for 1.5 h [14]. A lipid precipitate was removed by centrifugation. The water-soluble portion was fractionated by gel chromatography on a column (65 × 1.6 cm) of Sephadex G-50 (S) (Pharmacia, Sweden) in 0.05 M pyridine acetate buffer, pH 4.5, with monitoring the elution by the reaction with phenol and sulfuric acid. As a result, a high-molecular-mass polysaccharide and a core oligosaccharide were obtained in yields of 34.1 and 10.2%, respectively.

Total sugar content was determined by the reaction with sulfuric acid and phenol. Proteins were detected by the method of Lowry. Phosphorus was determined by the reaction with ascorbic acid and ammonium molybdate after mineralization of the sample. Kdo was determined by the reaction with thiobarbituric acid. Heptoses were detected by the reaction with cysteine and sulfuric acid. The analyses were performed as described [13].

For sugar analysis, the LPS, OPS, and core oligosaccharide were hydrolyzed with 2 M trifluoroacetic acid (120°C, 2 h). After evaporation, monosaccharides were reduced with $NaBH_4$, acetylated with Ac_2O in pyridine, and analyzed by GLC using a Hewlett-Packard 5880 chromatograph (USA) equipped with a capillary column of Ultra 2 stationary phase, and by GLC/MS using a Hewlett-Packard 5989 instrument (USA) on a capillary column of HP-1 stationary phase. The absolute configurations of the monosaccharides were determined by GLC of the acetylated glycosides with (–)-2-octanol [15, 16].

Amino components (amino sugars, ethanolamine, phosphoethanolamine, and amino acids) were determined using an AAA-339 amino acid analyzer (Czech Republic) on a column (3.7 × 350 mm) of Ostion LG ANB cation-exchange resin in a stepwise gradient of standard sodium-citrate buffers at 50-65°C. Fatty acids composition of the LPS and isolated lipid A was determined as described [17]. Prior to the analysis, the LPS was treated with methanol and chloroform for removal of a free phospholipid contamination.

Methylation of the OPS was performed with MeI in dimethylsulfoxide in the presence of solid NaOH [18]. The methylated OPS was isolated by extraction with ethyl acetate and then hydrolyzed. Partially methylated monosaccharides were converted into the alditol acetates and analyzed by GLC and GLC-MS as described above.

NMR spectra were run on a Bruker DRX-500 spectrometer (Germany) for a solution in D_2O (99.96%) at 53°C (internal standard acetone, δ_H 2.225 ppm, δ_C 31.45 ppm). Prior to measurements, samples were freeze-dried twice from D_2O. Two-dimensional spectra were recorded using standard Bruker software; data were acquired and processed using the XWINNMR 2.1 program (Bruker, Germany). Mixing times of 120 and 200 msec were used in TOCSY and NOESY experiments, respectively.

Preparation of immune serum, ring precipitation, precipitation in agar by Ouchterlony, passive hemagglutination, immunoelectrophoresis, and ELISA were performed as described earlier [14].

RESULTS AND DISCUSSION

An LPS preparation isolated from bacterial cells of *P. syringae* pv. *atrofaciens* IMV 948 by a special mild procedure (washing out with 0.85% NaCl solution) contained 45% of carbohydrates (as determined by the reaction with phenol and sulfuric acid) and a small amount of protein (about 1.9% as determined by the method of Lowry). These data confirmed the known peculiar features of the LPS of *P. syringae* [18], namely, a high content in and loose binding to the outer membrane.

The isolated LPS showed a weak toxicity in D-galactosamine-sensitized mice of the BALB/c line. LD_{50} was 2.5 μg per mouse compared to 0.14 μg per mouse for LPS of *Escherichia coli* O55: B5 (Sigma, USA) used as positive control.

Injection of LPS in a concentration of 1 mg/ml into plant tissue (early and late cabbage, tomato) induced formation of the specific water-saturated spots on tomatoes and protuberances on cabbage leafs, but the reaction was weak (1-2 points).

Methanolysis of the LPS followed by GLC analysis revealed methyl esters of fatty acids (Fig. 1), including 2-hydroxy- and 3-hydroxyalcanoic acids, which are characteristic for pseudomonads [19]. GLC analysis of the alditol acetates derived from the LPS resulted in identification of Rha, Glc, and Gal in the ratios of 93.3 : 6.2 : 0.4.

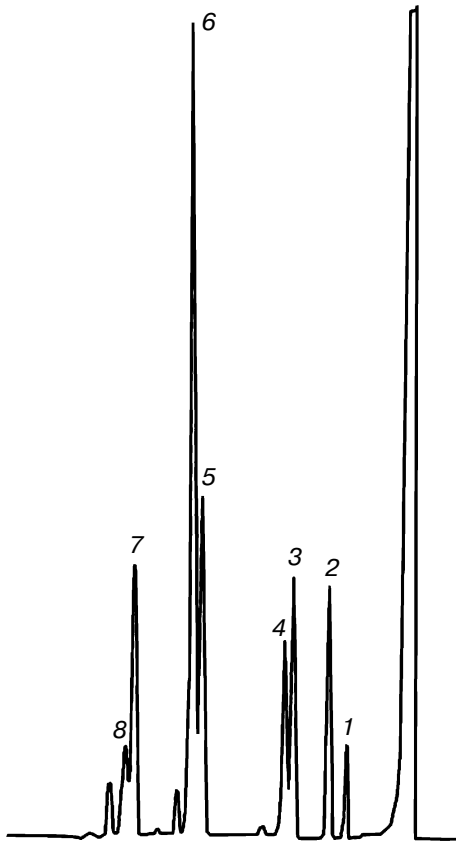

Fig. 1. GLC-profile of fatty acid methyl esters (peaks *1-8*) obtained from lipid A of *P. syringae* pv. *atrofaciens* IMV 948: *1*) 3-HO-$C_{10:0}$; *2*) $C_{12:0}$; *3*) 2-HO-$C_{12:0}$; *4*) 3-HO-$C_{12:0}$; *5*) $C_{16:1}$; *6*) $C_{16:0}$; *7*) $C_{18:1}$; *8*) $C_{18:0}$.

In addition, GlcN (5.1% of dry LPS weight), GalN (0.7%), alanine (1.7%), ethanolamine (1.1%), phosphoethanolamine (1.4%), Kdo (0.8%), and heptose (1.6%) were detected by ion-exchange chromatography on the amino acid analyzer and by colorimetric analysis.

Therefore, such characteristic components as hydroxylated fatty acids, Kdo, and heptose were found in the LPS studied. As in the LPS of *P. syringae* strains studied earlier [19], the main neutral sugar present is rhamnose.

Fractions of lipid A (53.4%), core oligosaccharide (10.2%), and OPS (34.1%) were obtained by mild acid hydrolysis of the LPS followed by gel chromatography on Sephadex G-50. Their ratios showed a predominance of the S-form LPS, which is also characteristic for other *P. syringae* strains [19], and, hence, the LPS of the studied strain has the macromolecular organization typical of this species.

In lipid A, fatty acids 3-HO-$C_{10:0}$, $C_{12:0}$, 2-HO-$C_{12:0}$, 3-HO-$C_{12:0}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$ were identified in the ratios 2.4 : 10.1 : 8.5 : 8.8 : 31.1 : 18.0 : 4.8 : 16.8, respectively. These data are close to those for other *P. syringae* [17, 19] and *Pseudomonas fluorescens* strains [20]. It should be noted that, in addition to fatty acids that are common for lipid A of the genus *Pseudomonas*, such as 3-HO-$C_{10:0}$, $C_{12:0}$, 2-HO-$C_{12:0}$, and 3-HO-$C_{12:0}$ [21], we found in all lipid A preparations studied nonhydroxylated fatty acids with a longer hydrocarbon chain, namely, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, and $C_{18:1}$. Their presence did not depend on the methods of extraction of the LPS preparations from the microbial cells and its purification (for example, treatment with chloroform and methanol for removal of phospholipids). They were not removed by dephosphorylation of the LPS with 48% hydrofluoric acid and were present not only in the cellular LPS, but also in the extracellular LPS isolated from a mixture of secondary metabolites, which were excreted to the environment by living microbial cells [20]. Earlier, fatty acids $C_{16:0}$ and $C_{18:0}$ have been identified by other authors in the LPS of *P. syringae* pv. *phaseolicola* [22] and *Pseudomonas aeruginosa* [23]. Based on these data, we suggest that saturated and unsaturated fatty acids $C_{16:0}$ and $C_{18:0}$ are the components of lipid A of pseudomonads.

GlcN, ethanolamine, phosphoethanolamine, and an unidentified amino sugar with the retention time T_{NH_3} 0.63 in amino acid analysis in the ratios of 5.9 : 1.4 : 0.7 : 2.3, respectively, were revealed as components of the hydrophilic part of lipid A. A number of amino acids were detected as well. Lipid A contained phosphate (1.4%) and neutral sugars (data of GLC analysis). Therefore, lipid A consists of characteristic components, but differs in the presence of an unidentified amino sugar and a low degree of phosphorylation.

In the core oligosaccharide, the following components were found: rhamnose, glucose (5 and 10% of dry weight, respectively; data of GLC of the alditol acetates), GlcN, GalN, alanine (4.6, 3.4, and 3.7%, respectively; data of amino acid analysis), L-*glycero*-D-*manno*-heptose, Kdo, and phosphate (4.4, 2.6, and 2.8% of dry weight, respectively; data of colorimetric analyses). The neutral sugars found (rhamnose, glucose, and heptose) are characteristic components of the LPS core of pseudomonads [13, 19, 20, 24]. An exception is the LPS of a *Pseudomonas fluorescens* strain [25], which lacks rhamnose, and, hence, this sugar is not an obligatory component of the LPS core of the genus *Pseudomonas*.

Composition and structure of the OPS were determined by acid hydrolysis, methylation, and NMR spectroscopy. Hydrolysis of the OPS with 2 M CF_3COOH followed by GLC analysis of monosaccharides as the alditol acetates resulted in identification of Rha and GlcN in the ratio of 4 : 1. GLC analysis of the acetylated glycosides with (–)-2-octanol showed that Rha has the L configuration and GlcN the D configuration.

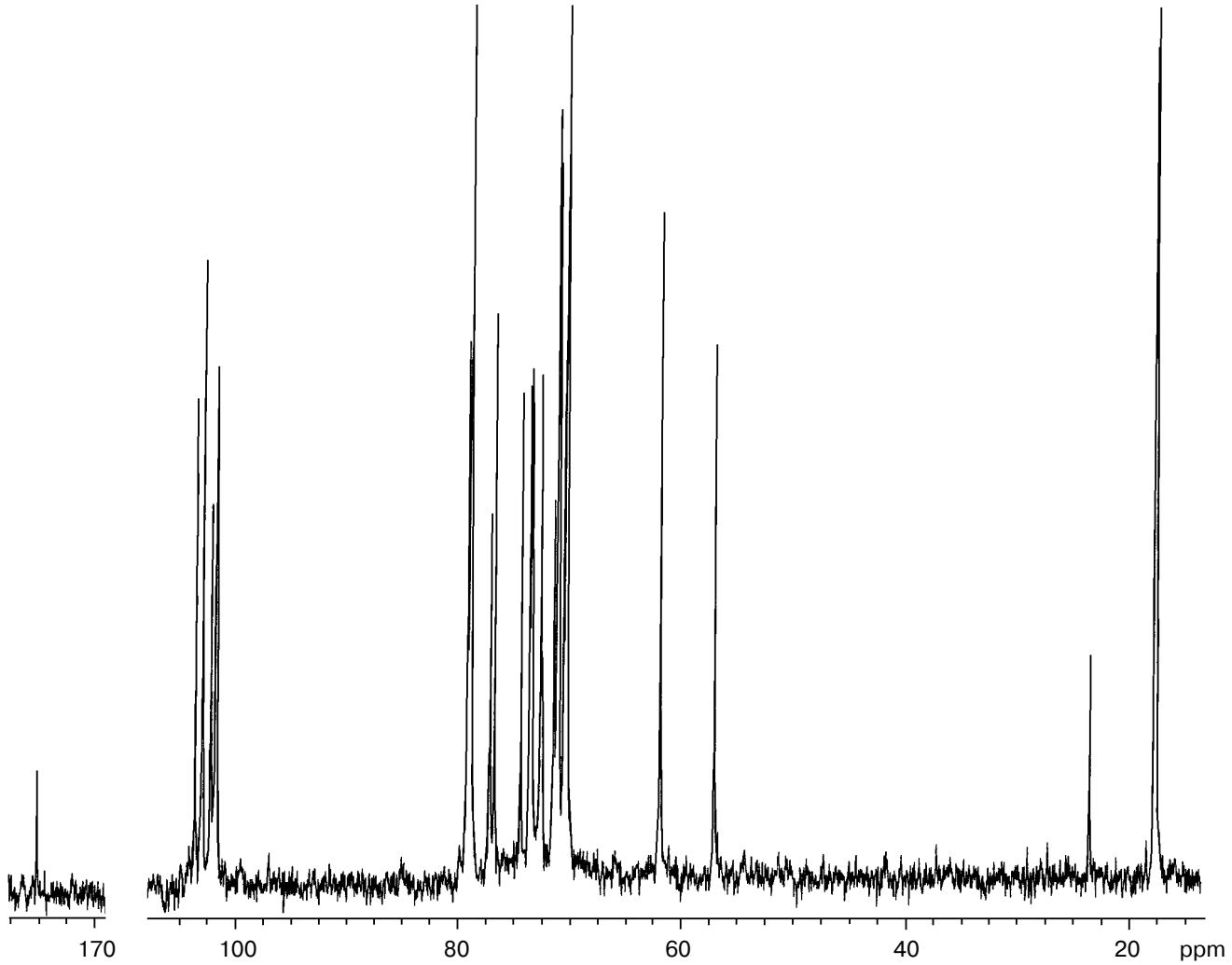

Fig. 2. ^{13}C-NMR spectrum of the O-specific polysaccharide of *P. syringae* pv. *atrofaciens* IMV 948.

The ^{1}H- and ^{13}C-NMR spectra of the OPS contained signals with different intensities, in which the major series for pentasaccharide repeating unit was clearly distinguished. Thus, in the ^{13}C-NMR spectrum (Fig. 2, Table 1), there were the major signals for five anomeric carbons at 101.8-103.7 ppm, four methyl groups (C6 of rhamnose) at 17.8-17.9 ppm, one nonsubstituted hydroxymethyl group (C6 of GlcN) at 62.0 ppm, one carbon bearing nitrogen (C2 of GlcN) at 57.1 ppm, 19 other sugar ring carbons linked to oxygen in the region 70.5-79.3 ppm, and one N-acetyl group at 23.6 (CH_3) and 175.2 (CO) ppm. Correspondingly, the ^{1}H-NMR spectrum (Fig. 3, Table 2) contained five signals for the anomeric protons of GlcNAc at 4.63 ppm (doublet, $J_{1,2}$ 8 Hz) and Rha at 4.98-5.24 ppm (four broadened singlets), four CH_3-C groups (H6 of Rha) at 1.28-1.34 ppm (doublet, $J_{5,6}$ 6-6.5 Hz), other sugar protons at 3.44-4.15 ppm, and one N-acetyl group at 2.09 ppm (singlet).

Thus, the OPS lacks a strict regularity, but consists mainly of pentasaccharide repeating units containing four L-Rha residues and one residue of D-GlcNAc. Methylation of the OPS followed by hydrolysis and GLC-MS analysis of the resultant partially methylated alditols revealed 2,4-di-O-methylrhamnose, 3,4-di-O-methylrhamnose, and 4-O-methylrhamnose in the ratios of ~1 : 2 : 1, as well as 2-deoxy-3,4,6-tri-O-methyl-2-(N-methyl)acetamidoglucose. Therefore, the OPS is branched and contains Rha residues substituted at position 2 or 3, rhamnose residue in the branching point substituted at positions 2 and 3, and a nonsubstituted terminal GlcNAc residue in the side chain.

The ^{1}H-NMR spectrum of the OPS (Table 2) was assigned using two-dimensional correlation spec-

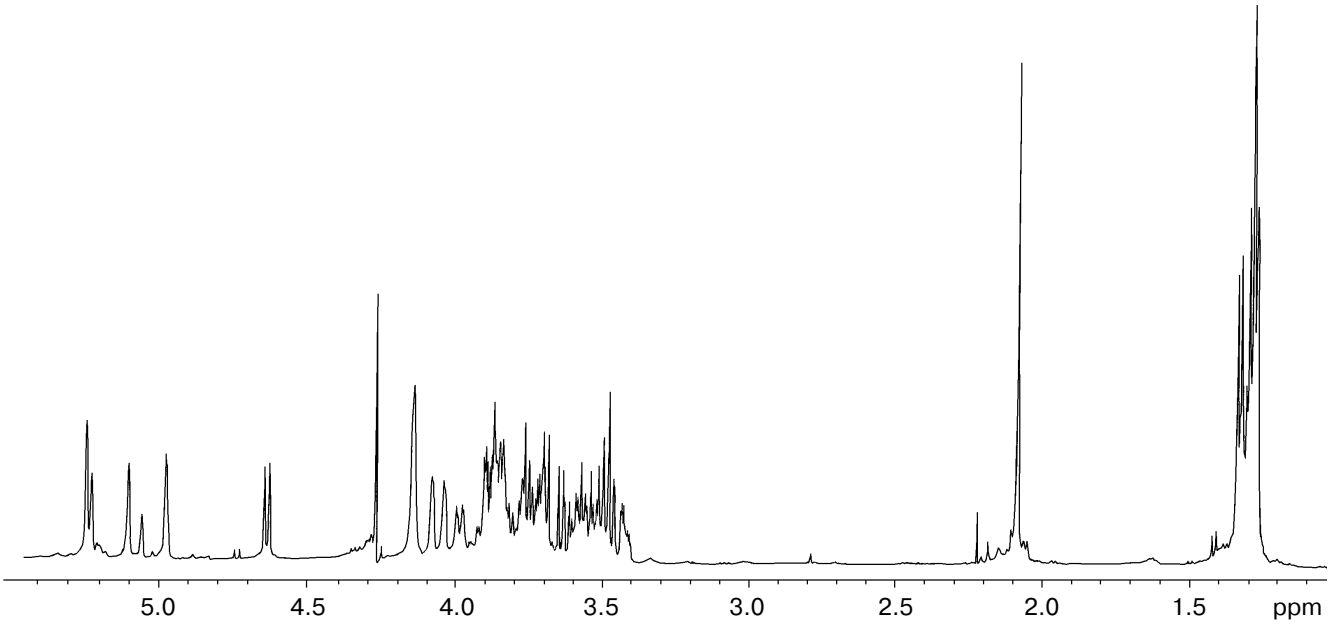

Fig. 3. ^{1}H-NMR spectrum of the O-specific polysaccharide of *P. syringae* pv. *atrofaciens* IMV 948.

troscopy (COSY) and total correlation spectroscopy (TOCSY). A $J_{1,2}$ coupling constant value of 8 Hz showed that the GlcNAc residue is β-linked. Accordingly, in the two-dimensional nuclear Overhauser effect spectrum (NOESY), interresidue H1/H3 and H1/H5 cross-peaks were present for the GlcNAc residue. All rhamnose residues (Rha^{I}-Rha^{IV}) showed interresidue cross-peaks H1/H2, and, hence, they have the α configuration.

With the ^{1}H-NMR spectrum assigned (Table 2), the ^{13}C-NMR spectrum of the OPS (Table 1) was assigned using a two-dimensional ^{1}H,^{13}C heteronuclear multiquantum correlation spectroscopy (HMQC). Signals for C3 of Rha^{I}, C2 and C3 of Rha^{II}, C2 of Rha^{III} and Rha^{IV} at 79.0, 79.1, 79.0 and 79.3 ppm, respectively, were shifted downfield, as compared to their position in the spectrum of the corresponding nonsubstituted monosaccharides at 71.4-71.8 ppm [26]. These displacements were caused by the α-effect of glycosylation [26] and revealed the position of substitution of the rhamnose residues in the OPS. Signals for GlcNAc were close to those for nonsubstituted β-GlcNAc [26], which was in agreement with the terminal location of this sugar in the side chain.

Table 1. Data from the ^{13}C-NMR spectra of the O-specific polysaccharide (chemical shifts in ppm)

Residue	Carbon					
	C1	C2	C3	C4	C5	C6
→3)-α-L-Rha*p*^{I}-(1→	103.0	71.1	79.0	72.7	70.6	17.8
→3)-α-L-Rha*p*^{II}-(1→	102.3	79.1	77.2	73.7	70.7	17.8
2 ↑						
→2)-α-L-Rha*p*^{III}-(1→	101.8	79.0	71.6	73.5	70.5	17.9
→2)-α-L-Rha*p*^{IV}-(1→	101.9	79.3	71.3	73.6	70.5	17.9
β-D-Glc*p*NAc-(1→	103.7	57.1	74.5	71.3	76.8	62.0

Note: The assignment of the C6 signals of the rhamnose residues could be interchanged. Signals of the N-acetyl groups are at 23.6 and 175.2 ppm.

Table 2. Data from the ^{1}H-NMR spectra of the O-specific polysaccharide (chemical shifts in ppm)

Residue	Hydrogen					
	H1	H2	H3	H4	H5	H6a; H6b
→3)-α-L-Rha*p*^{I}-(1→	4.98	4.15	3.84	3.58	3.77	1.28
→3)-α-L-Rha*p*^{II}-(1→ 2 ↑	5.24	4.14	3.99	3.53	3.82	1.30
→2)-α-L-Rha*p*^{III}-(1→	5.23	4.04	3.85	3.54	3.71	1.34
→2)-α-L-Rha*p*^{IV}-(1→	5.10	4.08	3.90	3.48	3.70	1.28
β-D-Glc*p*NAc-(1→	4.63	3.70	3.64	3.48	3.44	3.76; 3.89

Note: A signal of the N-acetyl group is at 2.09 ppm.

The NOESY spectrum of the OPS showed interresidue cross-peaks between the following transglycosidic protons: Rha^{II} H1/Rha^{I} H3, Rha^{III} H1/Rha^{II} H3, Rha^{IV} H1/Rha^{III} H2, Rha^{I} H1/Rha^{IV} H2, and GlcNAc H1/Rha^{II} H2 at 5.24/3.84, 5.23/3.99, 5.10/4.04, 4.98/4.08, and 4.63/4.14 ppm, respectively. In addition, in the spectrum there were interresidue cross-peaks for Rha^{III} H1/Rha^{IV} H5 and Rha^{IV} H1/Rha^{I} H5 at 5.23/3.70 and 5.10/3.77 ppm, respectively, which were characteristic for α1→2-linked rhamnose disaccharides [27]. These data were in agreement with a branched structure of the OPS and the positions of substitution of the monosaccharide residues, which were established by methylation analysis and the ^{13}C-NMR spectroscopy (see above). They enabled also determination of the monosaccharides sequence in the OPS. The GlcNAc H1/Rha^{II} H2 cross-peak in the NOESY spectrum indicated the spatial closeness of the two protons of the non-linked monosaccharide residues.

Therefore, the major repeating unit of the O-specific polysaccharide of *P. syringae* pv. *atrofaciens* IMV 948 has the following structure:

→2)-α-L-Rha*p*^{IV}-(1→2)-α-L-Rha*p*^{III}-(1→3)-

β-D-Glc*p*NAc
1
↓
2
-α-L-Rha*p*^{II}-(1→3)-α-L-Rha*p*^{I}-(1→

From the monosaccharide residues present in the OPS in a lesser amount, ^{1}H-NMR signals of only one Rha residue could be assigned (H1-H6 at 5.06, 4.15, 3.92, 3.60, 3.88, and 1.33 ppm, respectively). As judged by the positions of the signals for C1 and C3 at 103.0 and 79.3 ppm, respectively, which were determined from the two-dimensional HMQC spectrum, this residue is substituted at position 3 and nonsubstituted at position 2. Signals for the other residues from the minor series could not be detected because of their coincidence with signals from the major series, and, therefore, the structure of the minor repeating unit could not be determined. Based on the relative integral intensities of the signals in the ^{1}H-NMR spectrum of the OPS, it was concluded that the major and minor repeating units occur in the ratio ~3.5 : 1.

Branched OPSs of *P. syringae* with four L-Rha residues in the main chain repeating unit represent a large group of structurally related heterogeneous polymers. The reason of the structural heterogeneity has been elucidated for polysaccharides studied earlier [10, 19, 27-32]. It is associated with the presence of two types of tetrasaccharide repeating units in the main chain. One of them, the major, contains two rhamnose residues substituted at position 2 and two rhamnose residues substituted at position 3. The other type, the minor, contains three rhamnose residues substituted at position 3 and one residue substituted at position 2. The established structure of the major repeating unit of the OPS of *P. syringae* pv. *atrofaciens* IMV 948 falls into this group, and it could be suggested that this polysaccharide contains also a minor repeating unit, which differs from the major repeating unit in substitution of Rha^{III} or Rha^{IV} at position 3. This suggestion was confirmed by identification of signals for Rha residues substituted at position 3 in the minor series of the ^{1}H-NMR spectrum, which were absent from the major series.

The fact that the OPS of *P. syringae* pv. *atrofaciens* IMV 948 is built up like OPSs of all other bacteria of this group, namely, contains a linear rhamnan as the backbone, from one hand, serves as a new example to confirm the regularity established earlier, and, on the other hand, demonstrates that strain IMV 948 is correctly placed to the species *P. syringae*. The accumulated data indicated the taxonomic importance of this character.

It is worth noting that the polysaccharide studied is not strictly regular, and, hence, the structural heterogeneity of the OPS from serogroup V was described for the first time. Earlier such type of heterogeneity was found in strains of *P. syringae* serogroup I [28-30] and some strains that were not included into the classification scheme [27, 31, 32]. Another type of structural heterogeneity of the OPS was described for some strains of *Ralstonia solanacearum* and *Burkholderia cepacia* [33-36].

Then, immunochemical studies of the LPS and its isolated fractions were performed. The LPS exhibited a high serological activity in the reactions with homologous O-antiserum raised by immunization of rabbits with heat-killed microbial cells. Titers in ring precipitation, passive hemagglutination, and ELISA were 1 : 500,000, 1 : 5,120, and 1 : 12,800, respectively. Two differently expressed precipitation lines were detected in double diffusion in agar and immunoelectrophoresis, whereas the LPS isolated from heat-killed microbial cells showed only one clear line (Fig. 4). Direct and cross-reactions of agglutination and precipitation by Ouchterlony using polyvalent and partially absorbed sera showed that the LPS studied has not less than three antigenic determinants. From them, only one, strongly pronounced determinant is heat-stable. Agglutination of microbial cells by polyvalent and cross-absorbed sera revealed a minor serological relationship between *P. syringae* pv. *atrofaciens* IMV 948 and *P. syringae* IMV K1025, IMV 2846, and IMV 2399, which also belong to pathovar *atrofaciens* and represent serogroups II, IV, and VI, respectively [5].

In inhibition of passive hemagglutination between O-antiserum against heat-killed microbial cells of *P. syringae* pv. *atrofaciens* IMV 948 and the homologous LPS, the OPS fraction exhibited a high serological activity, though it was slightly weaker than the activity of the LPS (4 and 1 μg/ml, respectively). In contrast, the core oligosaccharide was characterized in this test system by a low serological activity (128-256 μg/ml), and, hence, the main immunodominant sites of the LPS are located on the OPS.

Although the OPS of *P. syringae* pv. *atrofaciens* IMV 948 has unique structure of the repeating unit, it shows some structural similarity to OPSs from some other *P. syringae* strains (Table 3). Thus, it has the same lateral substituent (GlcNAc) as the OPSs of serogroups IV and VIII, but differs in the structure of the main rhamnan chain. The OPS of *P. syringae* pv. *ribicola* NCPPB 1010 demonstrates a higher degree of structural similarity: it differs from the OPS studied only in the site of attachment of the lateral GlcNAc residue.

ELISA with polyvalent antisera against heat-killed microbial cells showed a minor serological relationship between *P. syringae* pv. *atrofaciens* IMV 948 and some strains of serogroups IV and VIII. Thus, the reaction in the system of O-antisera against whole microbial cells of *P. syringae* pv. *atrofaciens* IMV 948/homologous LPS was inhibited at a concentration of 200 μg/ml only by the LPS of *P. syringae* pvs. *glycinea* IMV L-25, *atrofaciens* ATCC 4394, and *tabaci* IMV 225 (by 20, 5, and 28%, respectively). These data showed that the minor serological rela-

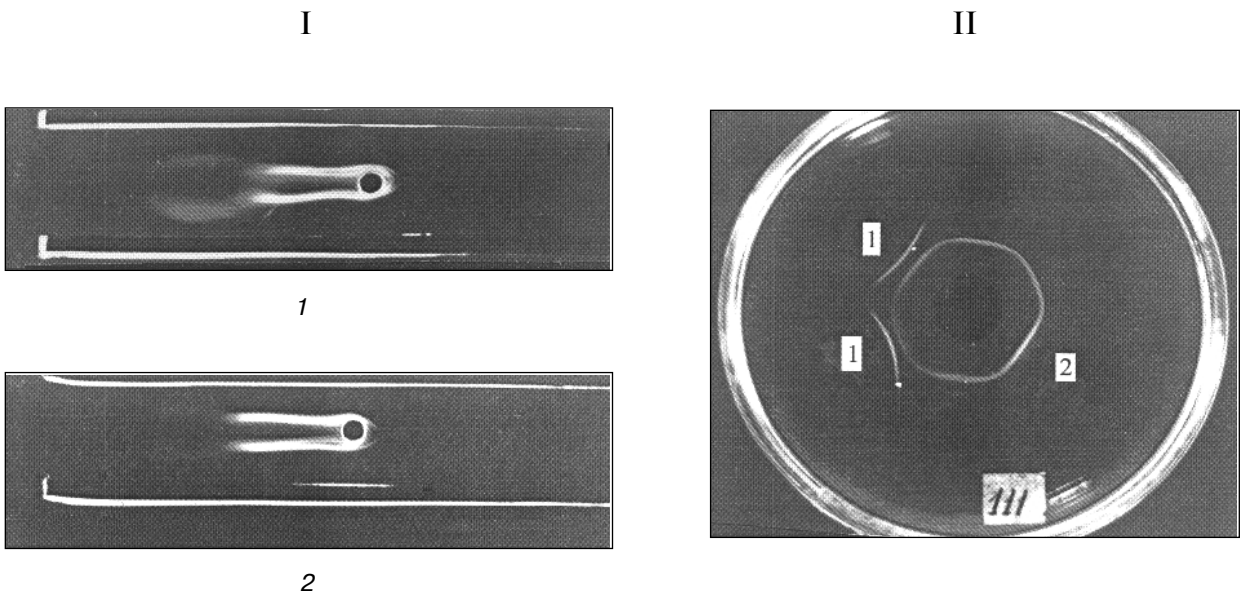

Fig. 4. Precipitation by Ouchterlony (I) and immunoelectrophoresis (II) of the LPS of *P. syringae* pv. *atrofaciens* IMV 948. *1*) LPS from living microbial cells; *2*) LPS from heat-killed microbial cells (2.5 h, 100°C).

Table 3. Structures of the OPSs from some strains of *P. syringae* having a structural similarity to the OPS of *P. syringae* pv. *atrofaciens* IMV 948

Pathovar and strain	Sero-group*	Structure of the repeating unit	Refe-rence
aptata 185 *atrofaciens* ATCC 4394 *atrofaciens* ATCC 2846 *glycinea* L-25 *lupini* 1234 *pisi* 7157 *syringae* (*holci*) 1055a *vignae* 7241 *wieringae* 7923	IV	β-D-Glc*p*NAc 1 ↓ 4 →3)-α-D-Rha*p*-(1→3)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→2)-α-D-Rha*p*-(1→	[37]
tabaci 225 *tabaci* P-28	VIII	β-D-Glc*p*NAc 1 ↓ 4 →3)-α-L-Rha*p*-(1→4)-β-L-Rha*p*-(1→3)-α-D-Rha*p*-(1→	[38]
ribicola NCPPB 1010**		β-D-Glc*p*NAc 1 ↓ 3 →2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→	[32]

* According to the classification scheme of Pastushenko and Simonovich [5].
** Strain is not included into the classification scheme. Only the structure of the major repeating unit is shown.

tionship observed is, most probably, due to antigenic determinants located on the core region of the LPS, rather than on the O-specific polysaccharide chain.

At the same time, neither polyvalent antisera nor monoclonal antibodies could reveal any LPS-based serological relationship between *P. syringae* pv. *atrofaciens* IMV 948 and *P. syringae* pv. *ribicola* NCPPB 1010 (V. V. Ovod, personal communication). These data indicated a serological inactivity of the rhamnan main chain in the presence of lateral sugar substituents. It could be suggested that the serological difference is defined by a different site of attachment of the side-chain β-GlcNAc residue to the main chain of the OPS of the two strains (GlcNAc is either (1→2)-linked to a rhamnose residue substituted at position 3 or (1→3)-linked to a rhamnose residue substituted at position 2, respectively). As a result, either different, non-cross-reactive epitopes are developed or conformational antigenic determinants on the OPS are changed [32]. Therefore, the unique structure of the OPS of *P. syringae* pv. *atrofaciens* IMV 948 defines the serological isolation of this strain.

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