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EXPERIMENTAL ARTICLES

Comparative Characterization of the Lipopolysaccharides of Different *Pseudomonas fluorescens* Biovar I Strains

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Abstract—From the biomass of five *Pseudomonas fluorescens* biovar I strains, including the *P. fluorescens* type strain IMV 4125 (ATCC 13525), lipopolysaccharides (LPS) were isolated (by extraction with a phenol–water mixture followed by repeated ultracentrifugation), as well as individual structural components of the LPS macromolecule: lipid A, the core oligosaccharide, and *O*-specific polysaccharide (*O*-PS). 3-Hydroxydecanoic, 2-hydroxydodecanoic, 3-hydroxydodecanoic, dodecanoic, hexadecanoic, octadecanoic, hexadecenoic, and octadecenoic fatty acids were present in lipid A of the LPS of all the strains studied. Glucosamine, ethanolamine, and phosphoethanolamine were revealed in the lipid A hydrophilic part of all of the strains. Glucose, rhamnose, mannoze, glucosamine, galactosamine, KDO, a trace amount of heptoses, ethanolamine, phosphoethanolamine, alanine, and phosphorus were identified as the main core components. Interstrain differences in the core oligosaccharide composition were revealed. Structural analysis showed that the O-PS of the type strain, as distinct from that of other strains, is heterogeneous and contains two types of repetitive units, including (1) three L-rhamnose residues (L-Rha), one 3-acetamide-3,6-dideoxy-D-galactose residue (D-Fuc3NAc) as a branching substitute of the L-rhamnan chain and (2) three L-Rha residues and two branching D-Fuc3NAc residues. The type strain is also serologically distinct from other biovar I strains due to the LPS *O*-chain structure, which is similar to those of the strains of the species *Pseudomonas syringae*, including the type strain. The data of structural analysis agree well with the results of immunochemical studies of LPS.

Key words: *Pseudomonas fluorescens*, lipopolysaccharide, O-specific polysaccharide, structure, lipid A, core oligosaccharide, serological cross reactivity.

Lipopolysaccharide (LPS) is a unique biopolymer of gram-negative bacteria. It is an important constructive element of the cell envelope and the main outer membrane antigen of gram-negative bacteria. LPS composition and structure are currently recognized as important chemotaxonomic criteria enabling researchers to determine phylogenetic relationships between bacteria and to trace back their evolutionary pathways, since individual structural parts of the LPS molecule, namely, the *O*-specific polysaccharide (*O*-PS), the core oligosaccharide, and lipid A, are different in structure and functions and exhibit different degrees of conservatism.

Being microbial endotoxins and *O*-antigens, LPS play a leading role in the infectious process; therefore, their study can provide insight into the mechanisms of interrelationships in the host–pathogen system. Proceeding from the foregoing, the study of the LPS of gram-negative bacteria arouses the unflagging interest of investigators.

We have been conducting systematic studies of the LPS of the widespread saprophytic bacteria of the species *Pseudomonas fluorescens*. In its physiological, biochemical, and genotypic characteristics, this is a heterogeneous group of bacteria whose strains are subdivided into five biovars in the *Bergey's Manual* [1]. At present, the taxonomic rank of individual biovars is subject to debate. Strain differences are noted within biovars as well. Numerical analysis fails to outline the subgroup of strains corresponding to biovar I described in the literature, which is the type biovar for the species. Except for several strains, the homology level of biovar I strains with the type strain of the species was not determined. Thus, at present, many problems concerning the taxonomy of the above-mentioned bacteria are to be solved. Their solution necessitates the use of chemotaxonomic criteria, which are usually more conservative than phenotypic properties. One of the chemotaxonomic criteria are the LPS properties.

P. fluorescens representatives are not infrequently isolated from clinical samples [2], but their possible pathogenicity remains to be elucidated. Considering

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Table 1. General characterization of the LPS preparations isolated from different *P. fluorescens* strains

Component (% of the of the dry preparation weight)	Strains				
	4125	472	1152	1433	7769
Carbohydrates[1]	36.6	42.0	38.0	50.0	16.5
Lipid A[2]	84.4	40.0	45.0	50.0	62.8
Protein[3]	2.8	3.0	0.6	1.5	1.4
Nucleic acids[4]	Traces	Traces	Traces	Traces	1.3
Phosphorus	2.9	4.0	3.4	5.0	4.5
Serological activity	+	+	+	+	+
Yield[5]	0.6	2.9	2.6	5.5	10.0

[1] Determined by the reaction with phenol and sulfuric acid.
[2] The precipitate formed after treating LPS with dilute acetic acid.
[3] According to Lowry method.
[4] According to Spirin's method.
[5] % of the dry bacterial mass weight.

the significant role of LPS in the infectious process, their study can make a certain contribution to the solution of this problem.

We have been studying the LPS of *P. fluorescens* strains assigned to biovar I. To date, we have characterized the LPS from five strains of this biovar at the structural–functional level [1, 3–9].

The aim of the present work was to carry out comparative analysis of the LPS of the type strain of *P. fluorescens* and other strains of biovar I, to characterize the LPS properties of the biovar strains on the whole, and to clear up the serological interrelationships among biovar I strains and with strains of other taxa.

MATERIALS AND METHODS

The subjects studied were *P. fluorescens* strains IMV 4125 (ATCC 13525), IMV 472, IMV 1152, and IMV 1433, which were obtained from E.A. Kiprianova (the collection of the Institute of Microbiology and Virology (IMV), National Academy of Sciences of Ukraine) and strain IMV 7769, which was identified and provided for study by L.A. Pasichnik.

The conditions for growing the bacteria and the procedures for the preparation of the microbial mass, LPS recovery and purification, and the obtaining of individual structural parts of the LPS macromolecule—lipid A, the core oligosaccharide, and *O*-specific polysaccharide (*O*-PS)—are described in [1, 3]. Neutral sugars were determined, after the acid hydrolysis of the samples, by GLC [3] as polyol acetates and by ion-exchange chromatography [7]. The amino compounds (amino sugars, ethanolamine, phosphoethanolamine, amino acids) were determined, after acid hydrolysis of the samples, on an amino acid analyzer [5]. The lipid A fatty acid composition was determined, after methanolysis of the samples, by GLC and GLC–mass spectrometry as described in [10]. The components of the hydrophilic part of lipid A macromolecule (amino sugars, ethanolamine, phosphoethanolamine, amino acids) were analyzed, after acid hydrolysis of the samples, on an amino acid analyzer. The structural analysis of the *O*-PS fractions was carried out as described earlier [4, 6, 9].

The sample contents of carbohydrates, protein, nucleic acids, KDO, heptoses, and phosphorus were determined as described earlier [1, 11].

The preparation of *O*-antisera and the methods of the serological studies were presented earlier [1, 3].

RESULTS AND DISCUSSION

The biopolymers isolated from the microbial mass of the strains studied by phenol–water extraction contained all the characteristic components of LPS (carbohydrates, lipid A, protein) (Table 1) and were active in the reactions of ring precipitation, double agar diffusion (DAD) according to Ouchterloni, and immunoenzyme assay (IEA) with the homologous *O*-serum.

As can be seen from the data presented, *P. fluorescens* type strain 4125 differs from other biovar I strains by a low LPS yield. Strain 7769 isolated as a rye phytopathogen [1] is characterized by a higher, as compared with the saprophytic strains, LPS content and its weak retention in the outer membrane (it can be washed out with a saline solution). This feature is also typical of phytopathogenic strains of the species *Pseudomonas syringae* [11] and may be related to the phytopathogenesic process, one of the symptoms of which is the obstruction of conducting vessels with substances of carbohydrate nature.

After treating LPS with 1% CH_3COOH, the structural parts of its macromolecule were obtained. The lipid A fraction was isolated as a water-insoluble precipitate. A distinctive feature of the type strain IMV 4125 was the highest content of this fraction (Table 1). The water-soluble products of LPS hydrolysis were separated on a column with Sephadex G-50. As can be seen from the elution profiles (Fig. 1), native LPS preparations of all strains were represented by a mixture of the S-and R-types of molecules, which is evidenced by the presence of the high-molecular-weight fraction of the *O*-specific polysaccharide (peak I) and the low-molecular-weight fractions of the core oligosaccharide (peaks II and III). Each of the strains was characterized by an individual polysaccharide elution profile. S-LPS prevailed in the LPS mixture of strain 4125; this is characteristic of the LPS of the *P. syringae* strains studied [12]. In most of the strains, R-LPS prevailed in the total preparation, as evidenced by a relatively high content of the core oligosaccharide fractions. The prevalence of R-LPS is characteristic of the LPS of the human pathogen *P. aeruginosa*; this, according to the literature data [13], may be linked to the peculiarities of the infection process caused by this bacterium. Thus, some strains of

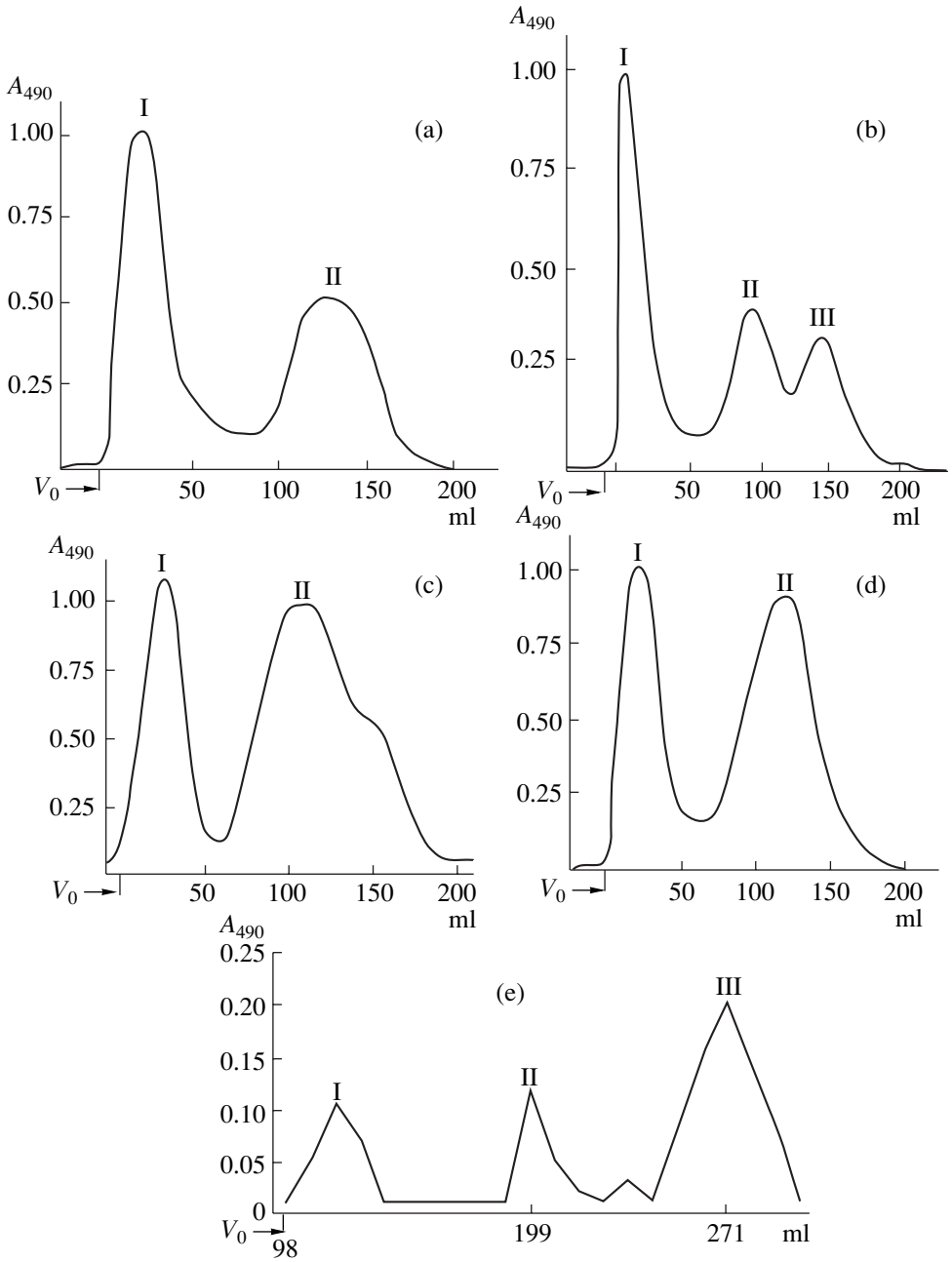

Fig. 1. Elution profiles of the LPS polysaccharides of (a) *P. fluorescens* IMV 4125, (b) IMV 472, (c) IMV 1152, (d) IMV 1433, and (e) IMV 7769 upon gel filtration on a column with Sephadex G-50: I is the fraction of *O*-specific polysaccharide; II and III are the fractions of the core oligosaccharide; V_0 is free volume.

the saprophytic species *P. fluorescens* are similar in this parameter to bacteria pathogenic to warm-blooded animals, and other strains are similar to phytopathogens.

Analysis of the fatty acid composition of lipid A fractions by GLC using columns with 5% SE-30, 5% DEGS-PS, and the corresponding markers of fatty acid methyl esters showed the presence of saturated and unsaturated fatty acids, as well hydroxy acids with a long carbon chain (from C_{10} to C_{18}), in the type strain and other strains of this biovar. The fatty acid methyl esters were identified using GLC–mass spectrometry. Interpretation of mass spectra was described in detail earlier [14].

The final results of qualitative and quantitative analysis of the lipid A fatty acid compositions of different *P. fluorescens* biovar I strains are shown in Table 2.

It can be seen from this table that dodecanoic ($C_{12:0}$), hexadecanoic ($C_{16:0}$), and octadecanoic ($C_{18:0}$) saturated fatty acids; hexadecenoic ($C_{16:1}$) and

Table 2. Fatty acid composition of lipid A of the LPS of different *P. fluorescens* biovar I strains (% of the total GLC peak area)

Fatty acid	Strains				
	4125	472	1152	1433	7769
2-OH-$C_{10:0}$	–	–	–	–	6.5
3-OH-$C_{10:0}$	7.8	9.5	23.4	6.8	11.5
$C_{12:0}$	14.8	4.6	7.4	6.6	16.5
2-OH-$C_{12:0}$	12.6	9.2	31.8	19.6	27.2
3-OH-$C_{12:0}$	12.0	26.0	28.7	27.1	6.8
$C_{16:1}$	13.3	25.5	1.7	8.0	4.5
$C_{16:0}$	23.7	12.6	3.4	17.9	20.2
$C_{18:1}$	13.0	11.3	2.6	6.9	3.7
$C_{18:0}$	2.9	1.3	0.5	1.2	3.0

Note: "–" denotes the absence of a fatty acid.

Table 3. Components of the hydrophilic part of lipids A

Component, %	Strains				
	4125	472	1152	1433	7769
Glucosamine	5.7	4.6	5.3	4.8	0.7
X	–	–	–	–	2.0
*X*1	–	–	–	–	0.3
*X*2	–	–	–	–	1.4
*X*3	–	–	–	–	2.2
Phosphoethanolamine	0.3	1.4	1.4	1.6	0.2
Phosphorus	2.7	3.2	3.0	4.2	1.2

Note: *X*, *X*1, *X*2, and *X*3 are unidentified amino compounds with retention times (relative to the standard (NH_3)) of 0.63, 0.65, 0.67, and 0.81, respectively; "–" denotes absence of the component.

octadecenoic ($C_{18:1}$) unsaturated fatty acids; 3-hydroxydecanoic (3-OH-$C_{10:0}$), 2-hydroxydodecanoic (2-OH-$C_{12:0}$), and 3-hydroxydodecanoic (3-OH-$C_{12:0}$) hydroxy acids revealed in the lipid A composition of the type strain occur in other biovar I strains as well. The hydroxy acids revealed in all of the strains often occur in typical pseudomonads (representatives of rRNA homology group I [10, 11]). Most *P. fluorescens* biovar I strains exhibited a high specific content of hydroxy acids (44.7 to 83.9%). The type strain IMV 4125, whose lipid A contained a relatively lower amount (32.4%) of hydroxy acids was an exception. Low content of hydroxy acids (13.4 to 24.0%) is characteristic of *P. syringae* lipids A [10, 11]. Thus, the type strain of *P. fluorescens* bears resemblance to *P. syringae* strains by this parameter as well. The biovar I strains significantly differed in the quantitative contents of particular fatty acids and virtually did not differ in their qualitative composition. 2-Hydroxydecanoic (2-OH-$C_{10:0}$) fatty acid was additionally detected only in strain IMV 7769. It should be pointed out that the attribution of hexadecenoic, octadecenoic, hexadecanoic, and octadecanoic acids to the pool of lipid A fatty acids is tentative due to the possible presence in the preparation of a mixture of phospholipids coextractable from the cells with hot phenol and due to possible partial degradation of other fatty acids [10]. However, we did not succeed in obtaining preparations lacking these fatty acids after the special treatment of LPS with methanol and chloroform to eliminate the possible admixture of phospholipids, as described earlier [10], and even after LPS dephosphorylation in the case of strain 472 [7].

Glucosamine and phosphoethanolamine were detected as the constituents of the hydrophilic part of the lipid A macromolecule (Table 3). The detection of glucosamine as the only amino sugar in four strains may indicate that the lipid A carbohydrate skeleton in the bacteria studied is represented by the residues of this sugar, which was the case in most of the gram-negative bacteria studied [10]. Strain IMV 7769, whose lipid A composition contained, in addition to glucosamine, unidentified amino compounds, was very distinctive among other strains. All lipids A are phosphorylated. Lipids A of the type strain IMV 4125, and strain IMV 7769 differed from lipids A of the other strains by their low phosphoethanolamine content.

Neither ion-exchange chromatography nor GLC revealed neutral sugars in the lipid A composition.

Thus, the strains studied are similar to each other in their lipid A fatty acid composition. The components of the hydrophilic part of the lipid A macromolecule are typical of the lipids A of most gram-negative bacteria. However, interstrain differences, which are indicative of the heterogeneity of the group of the bacteria studied, were also revealed.

Neutral sugars (identified by GLC in the form of polyol acetates), amino compounds (determined on an amino acid analyzer), and carbohydrates (determined by their specific reactions) (Table 4) were found to be the constituents of the core oligosaccharide fraction obtained by gel filtration on Sephadex G-50 (Fig. 1, peak I). Part of them (glucose, glucosamine, KDO, heptoses, ethanolamine, phosphoethanolamine, and phosphorus) are the characteristic components of this part of the LPS macromolecule in most gram-negative bacteria [15]; rhamnose, glucose, galactosamine, glucosamine, and alanine are often present in the core oligosaccharide of pseudomonads [11, 12, 15]. It should be pointed out that, of neutral sugars, only glucose had been found in the only *P. fluorescens* LPS core characterized at the structural level [16]. As seen from the comparative data presented, the LPS core of *P. fluorescens* biovar I strains contained not only rhamnose, but also sugars that are not typical of this part of the LPS macromolecule, such as fucose, arabinose, mannose, and galactose. In addition to glucosamine and galactosamine (amino sugars common for the LPS core region), other

amino sugars, e.g., *N*-acetyl fucosamine and N-acetyl quinovosamine, were present in the only *P. fluorescens* LPS core characterized at the structural level. In the bacteria studied in the present work, other amino sugars (unidentified) were revealed only as the core constituents of strain IMV 7769. All this may be evidence in favor of the possibility of the occurrence of different structural core types in the bacterial group studied. Several structural types of the LPS core oligosaccharide have been described in *P. aeruginosa* [17]. All the *P. fluorescens* strains studied were characterized by a low content of the inner core components (KDO and heptoses). The cores were rich in phosphorus, similar to the only *P. fluorescens* LPS core characterized at the structural level [16]. Individual strains differed in the qualitative composition of their core components and in their quantitative ratio.

We determined the core oligosaccharide heterogeneity of the bacteria studied. Thus, as seen from the HPLC elution profile of the strain IMV 472 core oligosaccharide on a μSpherogel-Carbohydrate column (Fig. 2), the preparation contained three components with different mobility, which seem to represent the core oligosaccharide fragments differing in the degree of completion of the biosynthesis process.

Thus, by the composition of the core, a phylogenetically conservative part of the LPS macromolecule [16], the strains studied represent a group of related, but not identical, bacteria.

The results of the analysis of the monosaccharide composition of the LPS *O*-PS fractions of different strains are given in Table 5.

It follows from the comparative data shown that sugars rarely occurring in nature, such as *D*-fucose, 3-acetamide-3-deoxy-D-fucose (the characteristic LPS components of *P. syringae* [11, 12, 18, 19]), and 4-acetamide-deoxy-D-fucose (revealed earlier only in two microbial polysaccharides, as a constituent of *Escherichia coli* O10 and as the common antigen of enterobacteria [4]), were present in the *O*-PS composition of the type strain 4125 and of the other biovar I strains. The simultaneous presence of the D- and L-isomers of *N*-acetyl quinovosamine in the LPS of the type strain is a unique feature. Thus, the type strain is unique among biovar I strains in the monosaccharide composition of the LPS *O*-chain. A total of three *O*-chain chemotypes were discovered in the strains studied.

Complete structural analysis of the LPS *O*-PS of the *P. fluorescens* biovar I strains, carried out using NMR spectroscopy, showed that the LPS *O*-PS of the type strain is not strictly regular. It follows from the structural analysis data that the *O*-PS of strain 4125 (ATCC 13525) of *P. fluorescens* is made up of two types of repetitive oligosaccharide units with structures **1** and **2** (Table 6) or is represented by two structurally related regular polysaccharides constructed of repetitive units

Table 4. Component composition of the core oligosaccharide fractions

Component	Strains				
	4125	472	1152	1433	7769
% of the total GLC peak area					
Rhamnose	19.8	9.0	24.6	19.0	27.4
Fucose	–	–	–	19.0	–
Arabinose	0.2	–	8.5	–	–≠
Mannose	18.0	–	2.3	–	–
Glucose	61.0	86.7	27.5	23.0	52.6
Galactose	Traces	3.7	ëÎ.	36.0	Traces
% of the dry preparation weight					
Glucosamine	1.0	9.5	3.1	10.5	2.2
Galactosamine	0.4	2.9	8.8	4.9	0.9
*X*1	–	–	–	–	6.9
*X*2	–	–	–	–	1.6
KDO	0.6	0.9	0.9	0.9	2.8
Heptoses	Traces	Traces	Traces	Traces	0.8
Ethanolamin	0.6	–	0.1	Traces	0.2
Phosphoethanolamine	0.8	0.7	0.7	0.7	0.9
Alanine	2.0	3.7	4.0	8.0	0.7
Phosphorus	3.6	3.5	3.9	4.8	7.8

Note: *X*1 and *X*2 are unidentified amino sugars with retention times relative to the standard (NH_3) of 0.86 and 0.88, respectively; "–" denotes absence of the component.

1 and **2**. Based on the rhamnose and Fuc3NAc ratio determined from data of GLC and NMR spectroscopy (the ratios of partially methylated rhamnose derivatives as well as the ratios of the oligosaccharides obtained after decomposition according to Smith), the repetitive

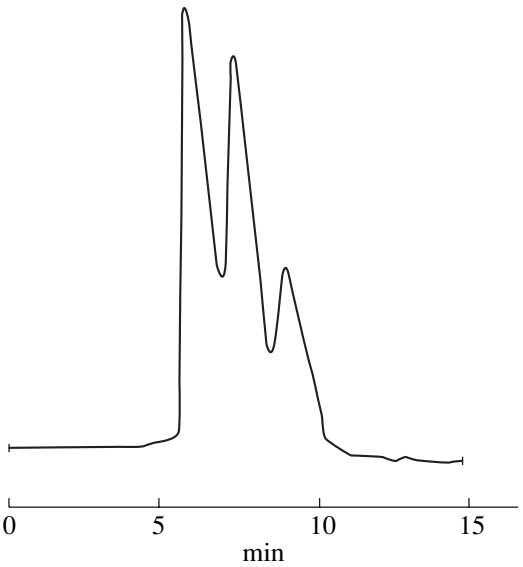

Fig. 2. HPLC-profile of the core oligosaccharide fraction on a μSpherogel-Carbohydrate (Beckman) column; eluent, H_2O; detector, a refractometer.

Table 5. Monosaccharide composition of *O*-PS (molar ratios)

Sugar residues (number)	Strains				
	4125	472	1152	1433	7769
L-Rha	2.5	2	–	–	–
D-Fuc	–	1	–	–	–
D-Fuc3NAc	1	–	–	–	–
D-Fuc4NAc	–	–	1	1	1
D-QuiNAc	–	–	1	1	1
L-QuiNAc	–	–	1	1	1
D-GlcNAc	–	1	–	–	–

Note: "–" denotes absence of the component. L-Rha, L-rhamnose; D-Fuc, D-fucose; D-Fuc3NAc, 3-acetamide-3-deoxy-D-fucose; D-Fuc4NAc, 4-acetamide-4-deoxy-D-fucose; D-QuiNAc, *N*-acetyl-D-quinovosamine; L-QuiNAc, *N*-acetyl-L-quinovosamine; D-GlcNAc, *N*-acetyl-D-glucosamine.

tetrasaccharide unit **1** constitutes approximately 60%. A similar LPS heterogeneity was also found in the LPS of many strains of *P. syringae* [12, 19, 20] *Burlkolderia cepacia* and *Ralstonia solanacearum* [21].

The biological expedience of such heterogeneity has not yet been understood, but it seems to be related to the adaptation of bacteria to the ever-changing environmental conditions.

As seen from the comparative data of the structural analysis of the *O*-PS of the *P. fluorescens* biovar I strains studied (Table 6), the heterogeneous *O*-PS of the type strain IMV 4125 (structures **1** and **2**) does not bear structural resemblance to the *O*-PS of the other biovar I strains. The comparison of the determined structure and the structures described earlier for the pseudomonad strains of other taxa showed a high degree of similarity between the *O*-PS of IMV 4125 and that of *P. syringae* strains (Table 7). Structure **1** (Table 6) of the *O*-PS of strain IMV 4125 is identical to that of the *O*-PS of *P. syringae* pv. *tabaci* IMV 223 strain (Table 7) and is similar (to a varying degree) to the *O*-PS of the strains shown in this table, including IMV 281, the type strain for *P. syringae.* It can be seen that the LPS *O*-PS of strain IMV 281 also demonstrates structural heterogeneity and is represented by two types of repetitive units (structures **3** and **4**).

To date, structure **2** (Table 6) of the IMV 4125 *O*-PS is unique.

Structure **3** of the *O*-PS of strain IMV 472 described has no counterpart either among *P. fluorescens* biovar I strains or among the other bacteria studied so far.

Table 6. Structure of the *O*-PS of *P. fluorescens* biovar I strains

α-D-Fuc*p*3NAc
1
↓
2
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

1

α-D-Fuc*p*3NAc α-D-Fuc*p*3NAc
1 1
↓ ↓
2 2
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

2

Strain IMV 4125 (type)

α-D-Glc*p*3NAc
1
↓
2
→3)-β-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-D-Fuc*p*-(1→

3

Strain IMV 472

→3)-β-L-Qui*p*NAc-(1 → 3)-β-D-Qui*p*NAc-(1 → 3)-α-D-Fuc*p*-4NAc-(1→

4

Strains IMV 1152, IMV 1433, and IMV 7769

Table 7. Structure of the *O*-PS of some *P. syringae* strains

α-D-Fuc*p*3NAc
↓2
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

1

(*P. syringae* pv. *tabaci* IMV 223) [18]

α-D-Fuc*p*3NAc
↓3
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

2

(*P. syringae* pv. *tomato* IMV 140R) [19]

α-D-Fuc*p*3NAc
↓3
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

3

α-D-Fuc*p*3NAc
↓3
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

4

(*P. syringae* pv. *syringae* IMV 281, IMV 460) [19]

α-D-Fuc*p*3NAc
↓3
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

3

α-D-Fuc*p*3NAc
↓3
→3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1 → 2)-α-L-Rha*p*-(1→

5

(*P. syringae* pv. *syringae* (*holci*) IMV 8300) [19]

α-D-Fuc*p*3NAc
↓3
→2)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1 → 3)-α-L-Rha*p*-(1→

6

α-D-Fuc*p*3NAc α-D-Fuc*p*3NAc
↓3 ↓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→

7

(*P. syringae* pv. *maculicola* IMV 381, pv. *tomato* 483) [20]

It is only structure **4** (Table 6) that proved common to three of the five *P. fluorescens* biovar I strains studied. Thus, three chemotypes of the LPS *O*-chains revealed by the analysis of the monosaccharide compositions corresponded to their three structural types. Structure **4** has not yet been described in the LPS of other bacteria. Proceeding from the literature data [22] on the LPS structure of the human pathogen *P. aeruginosa*, structure **4** mimics the LPS of *P. aeruginosa* by its general architectonics and by the nature of the monosaccharide residues present in it (linear aminoglycan); this may indicate the possibility of the clinical significance of some *P. fluorescens* strains. The data on the presence of antibodies against the LPS of strain IMV 7769 in the blood serum of patients with gastric ulcer and chronic nephritis in the exacerbation period [1] support this suggestion, although strain IMV 7769 was originally isolated as a rye pathogen.

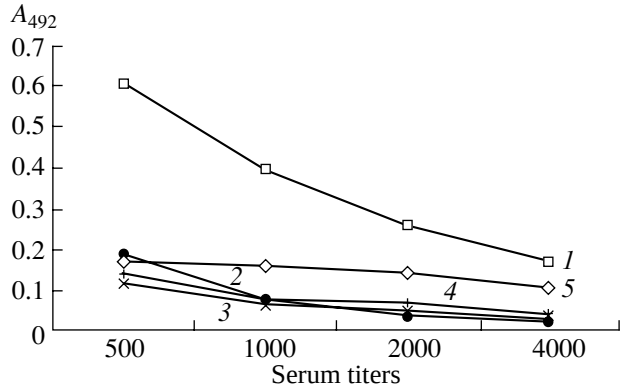

Fig. 3. Reactions (IEA) of the *O*-antiserum against *P. fluorescens* IMV 4125 with (*1*) homologous LPS and LPS from (*2*) *P. fluorescens* IMV 1152, (*3*) IMV 1433, (*4*) IMV 7769, and (*5*) IMV 472.

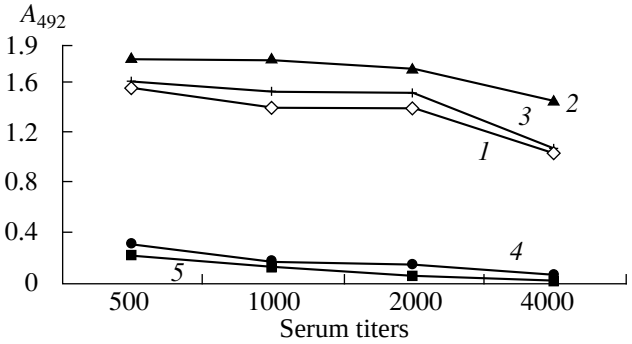

Fig. 4. Reactions (IEA) of the *O*-antiserum against *P. fluorescens* IMV 7769 with (*1*) homologous LPS and LPS from (*2*) *P. fluorescens* IMV 1152, (*3*) IMV 1433, (*4*) IMV 4125, and (*5*) IMV 472.

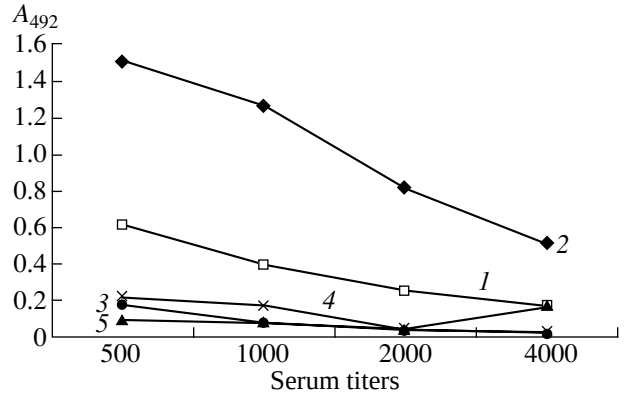

Fig. 5. Reactions (IEA) of the *O*-antiserum against *P. fluorescens* IMV 4125 with (*1*) homologous LPS and structurally similar LPS from *P. syringae* (*2*) pv. *tabaci* IMV 223, (*3*) pv. *syringae* IMV 281, (*4*) pv. *syringae* (*holci)* IMV 8300, and (*5*) pv. *tomato* IMV 140 R.

The data of structural analysis correlated well with the results of immunochemical LPS studies. In the IEA reaction of LPS of different strains with strain IMV 4125 antiserum (Fig. 3), the serological isolation of the type strain from biovar I strains was ascertained. Obviously, weak cross reactions with the LPS of the other strains occur due to the common antigenic epitopes located in the core and lipid parts of the LPS macromolecule.

The IEA reaction with the serum against strain IMV 7769 (Fig. 4) revealed close serological affinity between strains IMV 7769, IMV 1152, and IMV 1433, exhibiting identical structure of the LPS *O*-chain and a low serological relatedness of the above strains and strains IMV 4125 and IMV 472, differing in the *O*-PS structure.

Being structurally and serologically isolated among *P. fluorescens* biovar I strains, the species type strain IMV 4125 manifested a very high degree of serological affinity to strain *P. syringae* pv. *tabaci* IMV 223 (Fig. 5), whose *O*-chain structure is identical with structure **1** (Table 6) occurring in the heterogeneous LPS of strain IMV 4125. Despite the significant structural similarity (Table 7) (L-rhamnan as the main component of the *O*-chain); the common branching substitute (D-Fuc3NAc); the same type of substitution of the rhamnose residues; and the configurations of glycoside bonds), the LPS of other *P. syringae* strains reacted weakly in IEA with the antiserum against IMV 4125. These data may be indicative of a very high degree of specificity of immunological reactions.

Thus, the LPS of the *P. fluorescens* type strain IMV 4125 (ATCC 13525) and the other studied biovar I strains (IMV 472, IMV 1152, IMV 1433, and IMV 7769), isolated from the dry bacterial mass by phenol–water extraction and purified by repeat centrifugation, contained lipid A, the core oligosaccharide, and *O*-PS, i.e., all the characteristic components of the macromolecule of these biopolymers. In the LPS pool of strain IMV 4125, R-type molecules prevailed, which is not characteristic of the other biovar I strains studied. Glucosamine, phosphoethanolamine, 3-hydroxydecanoic, 2- and 3-hydroxydodecanoic, dodecanoic, hexadecanoic, octadecanoic, hexadecenoic, and octadecenoic fatty acids were identified as lipid A constituents in all the strains. All these components are also typical of lipids A from the other strains of the true pseudomonads so far studied. All the main components of the core oligosaccharide were found in the *P. fluorescens* biovar I strains studied. The type strain differed in the structure of the LPS *O*-chain from the other *P. fluorescens* biovar I strains studied, which correlates with the absence of serological relationships revealed in the IEA reaction. A varying degree of structural similarity was revealed between the *O*-PS of this strain and those of *P. syringae* strains, which correlates with the serological interrelationships revealed in the IEA reaction. Thus, the *P. flu-*

orescens type strain IMV 4125 is also serologically isolated from the biovar I strains and other strains of this species in the LPS structural properties and bears significant resemblance in these features to *P. syringae* strains, including the *P. syringae* type strain IMV 281.

According to the structural peculiarities of LPS and their serological properties biovar I of *P. fluorescens* is a heterogeneous group of bacteria. The interstrain relationships are best revealed at the level of the composition and structural specific features of lipid A, phylogenetically the most conservative part of the LPS macromolecule.

Three of five *P. fluorescens* biovar I strains studied have an identical LPS *O*-chain structure and bear similarity in the general architectonics of the molecule and in the peculiarities of its composition to the LPS of the human pathogen *P. aeruginosa*.

REFERENCES

1. Zdorovenko, G.M., Gvozdyak, R.I., Gubanova, N.Ya., Afonina, G.B., and Zdorovenko, E.L., Characterization of the Lipopolysaccharide from a *Pseudomonas fluorescens* Biovar I Strain, *Mikrobiologiya*, 1999, vol. 68, no. 3, pp. 330–339.

2. Gravents, Z.A., Ecology, Clinical Significance and Antimicrobial Susceptibility of Infrequently Encountered Glucose-Nonfermenting Gram-Negative Rods, *Nonfermentative Gram-Negative Rods, Microbiol. Ser.*, Gilardi, G.L., Ed., New York, 1985, vol. 16, pp. 131–233.

3. Zdorovenko, G.M., Veremeichenko, S.N., and Zakharova, I.Ya., Comparative Characterization of the Lipopolysaccharides of Various *Pseudomonas fluorescens* Strains, *Mikrobiol. Zh.*, 1987, vol. 49, no. 5, p. 13.

4. Knirel, Yu.A., Paramonov, N.A., Shashkov, A.S., Kochetkov, N.K., Zdorovenko, G.M., Veremeichenko, S.N., and Zakharova, I.Ya., Somatic Antigens of Pseudomonads: Structure of the *O*-Specific Polysaccharide of *Pseudomonas fluorescens* Biovar A Strain IMV 1152, *Carbohydr. Res.*, 1993, vol. 243, p. 205.

5. Veremeichenko, S.N. and Zdorovenko, G.M., Characterization of the Lipopolysaccharide of *Pseudomonas fluorescens*, *Mikrobiologiya*, 1994, vol. 63, no. 5, pp. 831–839.

6. Knirel, Yu.A., Veremeichenko, S.N., Zdorovenko, G.M., Shashkov, A.S., Paramonov, N.A., Zakharova, I.Ya., and Kochetkov, N.K., Somatic Antigens of Pseudomonads: Structure of the *O*-Specific Polysaccharide of *Pseudomonas fluorescens* Biovar A Strain IMV 472, *Carbohydr. Res.*, 1994, vol. 259, p. 147.

7. Veremeichenko, S.N. and Zdorovenko, G.M., Characterization of the Lipopolysaccharide of *Pseudomonas fluorescens* IMV 472 (Biovar I), *Mikrobiologiya*, 1996, vol. 65, no. 3, pp. 318–325.

8. Veremeichenko, S.N., Characterization of the Lipopolysaccharide of *Pseudomonas fluorescens* IMB 1433 (Biovar I), *Mikrobiologiya*, 1998, vol. 67, no. 4, pp. 505–511.

9. Knirel, Yu.A., Zdorovenko, G.M., Paramonov, N.A., Veremeichenko, S.N., Toukach, F.V., and Shashkov, A.S., Somatic Antigens of Pseudomonads: Structure of the *O*-Specific Polysaccharide of the Reference Strain for *Pseudomonas fluorescens* (IMV 4125, ATCC 13525, Biovar A), *Carbohydr. Res.*, 1996, vol. 291, p. 217.

10. Zdorovenko, G.M., Veremeichenko, S.N., and Solyanik, L.P., Fatty Acid Composition and Components of the Hydrophilic Part of Lipid A in the Lipopolysaccharide from *Pseudomonas syringae*, *Biochemistry* (Moscow), 1995, vol. 60, no. 7, pp. 787–793.

11. Zdorovenko, G.M., Solyanik, L.P., Yakovleva, L.M., and Paramonov, N.A., Characterization of *O*-Antigens from Different Strains of *Pseudomonas syringae* pv. *tabaci*, *Biochemistry* (Moscow), 1997, vol. 62, no. 1, pp. 28–37.

12. Zdorovenko, G., Gubanova, N., Solyanik, L., Knirel, Yu., Yakovleva, L., and Zakharova, I., Composition and Structure of Lipopolysaccharides from the Strains of Different Pathovars of *Pseudomonas syringae*, *Proc. 4th Int. Working Group on Pseudomonas syringae Pathovars, Stamperia Granducale, Florence*, 1998, pp. 391–401.

13. Meadow, P.M., Rowe, P.S., and Wells, P.L., Characterization of Polyagglutinating Antigens in *Pseudomonas aeruginosa*, *J. Gen. Microbiol.*, 1984, vol. 130, no. 2, pp. 631–644.

14. Veremeichenko, S.N., Zdorovenko, G.M., and Zakharova, I.Ya., Fatty-Acid Composition of Lipid A in *Pseudomonas fluorescens*, *Mikrobiologiya*, 1989, vol. 58, no. 2, pp. 229–235.

15. Knirel', Yu.A. and Kochetkov, N.K., The Structure of Lipopolysaccharides of Gram-Negative Bacteria: II. Structure of the Core (A Review), *Biokhimiya*, 1993, vol. 58, no. 2, pp. 182–201.

16. Knirel, Yu.A., Helbig, J.H., and Zahringer, U., Structure of a Decasaccharide Isolated by Mild Acid Degradation and Dephosphorylation of the Lipopolysaccharide of *Pseudomonas fluorescens* Strain ATCC 49271, *Carbohydr. Res.*, 1996, vol. 283, pp. 129–139.

17. Masoud, Y., Sadovskaya, I., Kievit, T., Altman, E., Richards, J.C., and Lam, J.S., Structural Elucidation of the Lipopolysaccharide Core Region of the O-Chain-Deficient Mutant Strain A28 from *Pseudomonas aeruginosa* Serotype O6 (International Antigenic Typing Scheme), *J. Bacteriol.*, 1995, vol. 177, no. 23, pp. 6718–6726.

18. Shashkov, A.S., Zdorovenko, G.M., Daeva, E.D., Yakovleva, L.M., Solyanik, L.P., Gvozdyak, R.I., Knirel', Yu.A., and Kochetkov, N.K., Antigenic Bacterial Polysaccharides: 37. The Structure of the Polysaccharide Chain of the Lipopolysaccharide of *Pseudomonas syringae* pv. *tabaci* (Serogroup VII), *Bioorg. Khim.*, 1990, vol. 16, pp. 90–97.

19. Knirel, Yu.A. and Zdorovenko, G.M., Structures of *O*-Polysaccharide Chains of Lipopolysaccharides as the

Basis for Classification of *Pseudomonas syringae* and Related Strains, *Pseudomonas syringae Pathovars and Related Pathogens*, Rudolph, K. *et al.*, Eds., Dordrecht: Kluwer Academic, 1997, pp. 475–480.

20. Knirel, Y.A., Ovod, V., Zdorovenko, G.M., Gvozdyak, R.I., and Krohn, K., A Novel Structure of the *O*-Polysaccharide and Immunochemical Relationships between the Lipopolysaccharides of *Pseudomonas syringae* Pathovar *tomato* and Pathovar *maculicola*, *Eur. J. Biochem.*, 1998, vol. 258, pp. 657–661.

21. Varbanets, L.D., Moskalenko, N.V., Kavun, E.M., Muras, V.A., and Zhitkevich, N.V., Investigations of Antigenic Activity of Lipopolysaccharides from *Burkolderia solanacearum*, *Biochemistry* (Moscow), 1996, vol. 61, no. 5, pp. 601–606.

22. Knirel', Yu.A. and Kochetkov, N.K., The Structure of the Lipopolysaccharides of Gram-Negative Bacteria: III. The Structure of *O*-Specific Polysaccharides (A Review), *Biokhimiya*, 1994, vol. 59, no. 12, pp. 1784–1852.