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EXPERIMENTAL ARTICLES

Characterization of the Lipopolysaccharide from *Rahnella aquatilis* 1-95

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Abstract—The lipopolysaccharide from the freshwater bacterium *Rahnella aquatilis* 1-95 has been isolated and investigated for the first time. The structural components of the lipopolysaccharide molecule, such as lipid A, core oligosaccharide, and O-specific polysaccharide, were isolated by mild acidic hydrolysis. In lipid A, 3-hydroxytetradecanoic and tetradecanoic acids were found to be the predominant fatty acids. In the core, oligosaccharide, galactose, arabinose, fucose, and an unidentified component were shown to be the major monosaccharides. The O-specific polysaccharide consists of a regularly repeating trisaccharide unit with the following structure: $\begin{matrix}\alpha\text{-D-Gal}p\text{-}(1\rightarrow 2)\text{-}\\ \rightarrow 3)\text{-}\alpha\text{-D-Fuc}p\text{-}(1\rightarrow 3)\text{-}\beta\text{-D-Gal}f\text{-}(1\rightarrow\end{matrix}$. Both acyl and phosphate groups have been shown to be responsible for the toxic and pyrogenic properties of the lipopolysaccharide of *R. aquatilis.*

Key words: lipopolysaccharide, *Rahnella aquatilis*, structure, biological activity.

In recent years, several new genera and species of enterobacteria have been described due to the improvement in traditional methods of microbial systematics and the development of fundamentally new ones. However, only a few of the more than a hundred species of the family *Enterobacteriaceae* described by the end of 1995 have attracted the consistent attention of researchers; other species, including *Rahnella aquatilis*, have been little studied. Formerly, the bacteria of the latter species were assigned to *Enterobacter agglomerans* biogroup G1. In 1976, based on the application of numerical taxonomy, a special group (group H2) was created to include certain enterobacters [1]. By using DNA–DNA hybridization, it was shown that these bacteria are markedly distinct from the other representatives of the family *Enterobacteriaceae* and can be assigned to a separate new genus and species, *Rahnella aquatilis* [2]. The genus *Rahnella* was named in honor of the microbiologist O. Rahn, who contributed significantly to the classification of enterobacteria and proposed the family name *Enterobacteriaceae* in 1937. The species epithet "aquatilis" reflects the main natural habitat of these bacteria, which were first isolated from water bodies in France and the United States [1, 2]. Later, *R. aquatilis* representatives were isolated from soil, mainly from cereal rhizosphere, and from clinical samples. In addition to France and the United States, these bacteria have also been isolated in Canada, the United Kingdom and other European countries, and Saudi Arabia. In Ukraine, *R. aquatilis* has been isolated from water bodies, soils, food, healthy people, and patients with acute intestinal infections. Strains of *R. aquatilis* are quite similar to strains of *E. agglomerans*, belonging to biogroup G1, in their phenotypic properties [3, 4]. They utilize Simmons citrate as their sole source of carbon, lack arginine dehydrolase and lysine- and ornithine decarboxylase activity, ferment lactose, sucrose, and rhamnose, do not degrade adonite, are heterogeneous in their ability to ferment salicin and amygdalin, and grow at 10–37°C but not at 41°C. There is no well-defined scheme for differentiation between strains belonging to the species *R. aquatilis* and *E. agglomerans*. The composition and structure of the lipopolysaccharide (LPS), the main component of the outer membranes in gram-negative bacteria, are known to be taxon-specific and are recognized as important chemotaxonomic criteria in microbial systematics. Individual components of the LPS molecule (lipid A, core oligosaccharide, and O-specific polysaccharide) show different rates of evolutionary variability and thus can be used to characterize taxa of different levels.

Since the lipopolysaccharide from *R. aquatilis* has not been investigated, the aim of this work was to study its composition, structural features, and some of its biological properties.

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MATERIALS AND METHODS

The study was carried out with the Uda River (Kharkov) isolate *Rahnella aquatilis* 1-95, kindly provided by S.I. Pokhil (Metchnikoff Institute of Microbiology and Immunology, Academy of Medical Sciences of Ukraine). The bacteria were cultivated on nutrient agar at 28°C for 24 h. Then, the cells were washed off, sedimented by centrifugation, and dehydrated by treatment with acetone and diethyl ether. Lipopolysaccharides (LPSs) were extracted from the dry biomass with a water–phenol mixture [5], and the water layer was dialyzed sequentially against tap and distilled water. Nucleic acids were removed by precipitation with trichloroacetic acid, and the purified LPS was lyophilized.

Assays of the nucleic acids, carbohydrates, protein, heptoses, and 2-keto-3- deoxyoctulosonic acid (KDO) were carried out as described earlier [6].

The structural components of the LPS macromolecule (the O-specific polysaccharide (O-SP), core oligosaccharide, and lipid A) were isolated by cleavage of the LPS macromolecule with 3% acetic acid (100°C, 2 h) after preliminary removal of phospholipid contamination by extraction with methanol and chloroform. The sediment of lipid A was collected by ultracentrifugation (25000 *g* for 40 min), and the supernatant was concentrated to 10 ml and fractionated on a column (70 × 3 cm) with Sephadex G-50 using 0.025 M pyridine–acetate buffer (pH 4.5) as the eluting agent.

The amino acids and amino sugars released after the hydrolysis of preparations with 6 N HC1 (100°C, 20 h) were assayed on a KLA-5 amino acid analyzer (Hitachi, Japan).

In order to determine the composition of the neutral monosaccharides, the preparations were hydrolyzed with 2 N HCl (100°C for 5 h) or 2 M CF_3CO_2H (120°C for 2 h) and analyzed in the form of acetate polyols by GLC either on a Chrom-5 chromatograph (Czech Republic) equipped with a flame-ionization detector and a column (3.0 mm × 1.2 m) with 3% neopentyl glycol succinate on Chromosorb W (80–100 mesh) at a temperature programmed to rise from 170 to 200°C at 5°C/min or on a Hewlett-Packard 5880 (United States) equipped with an Ultra 2 capillary column (the temperature was 180°C for 1 min and then increased to 290°C at 10°C/min). The absolute configurations of the monosaccharides were determined by GLC of acetylated glycosides with the use of (S)-2-octanol on a Hewlett-Packard 5880 (United States) at 230°C (20 min).

The polysaccharide was methylated by treatment with CH_3I in dimethyl sulfoxide in the presence of methylsulfinyl methanide and then hydrolyzed by treatment with 2 M CF_3CO_2H at 100°C for 2 h. The partly methylated monosaccharides were reduced with $NaBH_4$, acetylated, and analyzed by GLC–mass spectrometry on a Hewlett-Packard 5880 (United States) equipped with a HP-5ms column; the column temperature was kept at 150°C for 3 min and then was raised to 320°C at a rate of 5°C/min.

The ^1H and ^{13}C NMR spectra of the preparations were recorded in D_2O at 27°C on a Bruker DRX-500 spectrometer (Germany). The chemical shifts were evaluated with the use of sodium-3-trimethylsilyl propanate-δ_4 (δ_H 0.00) and acetone (δ_C 31.45) as internal standards. Times of 200 and 150 ms were used in the processes of total correlation spectroscopy (TOCSY) and nuclear Overhauser effect spectroscopy (NOESY), respectively.

The fatty acid composition of the preparations was determined on a Chrom-5 gas chromatograph (Czech Republic) equipped with a flame-ionization detector and a column (3.0 mm × 1.2 m) packed with SE-30. Helium was used as the carrier gas, and the programmed temperature range, from 130 to 235°C, was scanned at 12°C/min.

In order to remove phosphate groups, the LPS samples were treated with 40% hydrofluoric acid at room temperature for 24 h. The mixture was then suspended in a small volume of water and adjusted to a neutral pH with the anion-exchange resin Dowex IX4 in CO_3^- form; then, the resin was removed by filtration through a glass filter, and the filtrate was frozen and lyophilized [7].

The *N,O*-deacylation of the LPS was performed by treatment of the preparations with anhydrous hydrazine in the presence of hydrazine sulfate at 100°C for 40 h. Then, the assay mixture was neutralized with 3 M HCl; free fatty acids were extracted with chloroform; and the aqueous fraction was dialyzed against distilled water, evaporated, frozen, and lyophilized [8].

Antiserum was obtained by immunization of 2.5-kg rabbits with increased doses of a heat-treated (100°C, 2.5 h) cell suspension of *R. aquatilis* (from 2×10^6 to 5×10^7 cells/ml). Immunization was performed four times at 7-day intervals. Blood samples were taken 7 days after the last injection. Double diffusion in agar gel was carried out according to the Ouchterlony method. Immunoelectrophoresis was applied in two modifications: microelectrophoresis and rocket electrophoresis [9].

The toxicity (LD_{50}) of the LPS was studied with the use of white mongrel mice sensitized with galactosamine.

The pyrogenic properties of the LPS were examined by intravenous injection of a predetermined minimal pyrogenic dose of the LPS into rabbits and subsequent thermometry of the animals for 3 h.

RESULTS AND DISCUSSION

The yield of LPS extracted from the *R. aquatilis* cells with a water–phenol mixture reached 7.7%, which is higher than the values typical of other gram-negative bacteria (up to 5%).

Table 1. Monosaccharide composition of the structural components of the LPS from *R. aquatilis* 1-95

Preparation	% of the sum of peak areas											% of dry preparation weight		
	Rhamnose	Fucose	Ribose	Arabinose	Xylose	Mannose	Galactose	Glucose	X_1	X_2	X_3	KDO	Heptoses	Glucosamine
LPS	–	12.3	2.0	0.2	0.3	0.3	78.7	2.3	0.9	3.0	–	0.95	Traces	0.85
O-PS	–	31.8	–	–	–	Traces	63.2	2.5	–	0.8	1.7	–	–	
Core oligosaccharide 2	11.1	–	–	–	–	Traces	44.0	5.4	37.6	Traces	1.9	–	26.6	
Core oligosaccharide 3	–	42.8	–	49.0	–	–	8.2	Traces	–	–	Traces	0.68	–	

Note: "–" means that a component is absent; X_1, X_2, and X_3 are unidentified monosaccharides with retention times higher than that of glucose.

The LPS from *R. aquatilis* was characterized by a rather high content of nucleic acids (30%), which might be due to the LPS extraction procedure applied. Nucleic acids were removed from the LPS preparations with a saturated solution of trichloroacetic acid, which forms insoluble complexes with nucleic acids. The purified LPS preparations contained carbohydrates (53.1%), proteins (0.6%), and nucleic acids (6.7%). Galactose was the dominant monosaccharide (78.7%). Fucose accounted for 12.3%, whereas the proportions of glucose, ribose, mannose, and several unidentified monosaccharides ranged from 0.2 to 3.0% (Table 1). KDO and heptoses, typical LPS components in gram-negative bacteria, were found in small (0.95%) and trace amounts, respectively. Hexosamines were represented by glucosamine (0.85%). Thus, the LPS isolated from *R. aquatilis* contained all the components typical of these biopolymers.

Until recently, it was believed that the amino acids revealed in LPSs result from contamination of the LPS preparations with proteins during the extraction procedure; however, in recent years, it has been inferred that amino acids are LPS constituents. In the units of the O-specific polysaccharide (O-PS) of *Proteus mirabilis* O27, noncarbohydrate substituents have been revealed: two amino acids, L-lysine and L-alanine, are linked through amide groups to the carboxyl groups of uronic acids, and ethanolamine is attached to the *N*-acetylglucosamine residue at the 6th position via a phosphodiester bond [10]. It was assumed that amino compounds play a role in the biological activity of LPSs. Indeed, the O-PS from *P. mirabilis* 028 has been found to contain L-serine and L-lysine, and a polyclonal antiserum has been shown to recognize the epitope containing D-Gal*p*A-Lys (but not D-Gal*p*A-Ser), in which galacturonic acid residue plays the immunodominant role [11].

In the LPS from *R. aquatilis*, amino acids were detected in insignificant amounts. The contents of serine, aspartic acid, glutamic acid, alanine, methionine, isoleucine, leucine, histidine, and lysine were 0.06, 0.05, 0.17, 0.07, 0.15, 0.10, 0.6, 0.5, and 0.07%, respectively; the prevalent amino acid was glycine (0.41%).

In order to isolate individual structural components of the LPS macromolecule, we applied mild acid hydrolysis (treatment with 3% acetic acid at 100°C for 2 h), which is known to cleave the ketoside bond between the KDO residue and hydroxyl group at the 6th carbon atom of the glucosamine II residue in the lipid A molecule; the precipitate of lipid A was separated by centrifugation. Lipid A is the most conservative moiety of the LPS molecule, as is indicated by the fact that it contains the antigenic epitopes responsible for cross reactions between remotely related taxa. Lipid A is a diphosphorylated β-1,6-linked glucosamine disaccharide whose OH-groups at positions 2, 2^1, 3, and 3^1 are linked, via amide or ester bonds, to fatty acids with chain lengths of 10 to more than 20. Lipids A from various bacterial species are known to differ in relation to the composition of fatty acids, which is a relatively stable characteristic and may serve as a chemotaxonomic criterion for the elucidation of phylogenetic relationships between microorganisms. The most diagnostically important are hydroxy acids, which are linked directly to the carbohydrate moiety of lipid A and are termed primary fatty acids, whereas fatty acids bound to them via the OH groups are termed secondary fatty acids. Analysis of the GLC profile of fatty acid methyl esters showed that the *R. aquatilis* lipid A contained fatty acids with a chain length from 10 to 18 (Fig. 1). Peaks 1, 3, 4, 6, 7, and 8 were identified, by comparison with standards, as saturated fatty acids, namely, dodecanoic (13.8%), tetradecanoic (19.9%), pentadecanoic (21.6%), hexadecanoic (11.3%), heptadecanoic (3.2%), and octadecanoic (13.3%) acids, respectively (Table 2). The retention time of peak 5 changed after trifluorine acetylation of the methyl ester mixture, which is characteristic of hydroxy acids. This peak was identified as 3-hydroxytetradecanoic acid, which is typical of *Enterobacteriaceae* representatives. This acid is linked to the carbohydrate moiety of lipid A through both amide and ester bonds. Its content in the *R. aquatilis* lipid A was 10.3%. C16:0 and C18:0 fatty acids, which we revealed in the LPS of *R. aquatilis*, were earlier found in the LPS

of *Ralstonia solanacearum* [12] and representatives of the genus *Pseudomonas* [13].

Lipid A is known to be the endotoxic center of the LPS molecule, which determines such LPS properties as lethal toxicity, pyrogenicity, Schwartzman reaction, the ability to induce the tumor necrosis factor and interleukines, adjuvant activity, and mitogenicity. The LPS from *R. aquatilis* 1-95 was less toxic to D-galactosamine-sensitized mice than the LPS from *Escherichia coli* 055:B5 (Sigma, United States), applied as a positive control: the LD_{50} values were 5.0 and 0.14 μg/mouse, respectively (Table 3). The pyrogenic effect of the LPS from *R. aquatilis* 1-95 was less pronounced than that of the pharmaceutical preparation Pyrogenal: the temperature changes were +0.70 and +0.59, +0.54 and +0.69, and +0.50 and +0.71°C 1, 2, and 3 h after the injections, respectively.

In order to determine the role of individual chemical groups of the lipid A molecule in the toxicity and pyrogenicity of the LPS, dephosphorylated and *N,O*-deacetylated forms of the LPS macromolecule were obtained. Unlike the native form, the modified LPS exhibited neither toxicity nor pyrogenicity (Table 3). Therefore, both acyl and phosphate groups appear to be responsible for the biological activity of the *R. aquatilis* LPS. However, it remains unclear which of the fatty acids are responsible for the toxic effect of the *R. aquatilis* LPS. The LPS from *Rhodocyclus gelatinosus* has been reported to be less toxic than the LPS from *E. coli*, in which both glucosamine residues carry fatty acids with greater chain lengths [14]. Similarly, the LPS from *R. solanacearum*, which contains short-chain fatty acids, are less toxic and pyrogenic than LPSs with longer chain fatty acids [15].

Apart from its composition, the endotoxic properties of lipid A are known to depend on the conformation of the molecule, which is determined by all of its components (fatty acids, phosphate groups, and the glucosamine disaccharide). The so-called endotoxic conformation corresponds to biologically active cubic and hexagonal supramolecular structures [16].

It is known that LPS preparations are always heterogeneous because the S forms of bacteria contain polysaccharides with different chain lengths and R-form molecules lacking polysaccharide chains. The heterogeneity of the *R. aquatilis* LPS is demonstrated by the presence of a high-molecular O-PS fraction (fraction *1*) and fractions *2* and *3* of the core oligosaccharide after gel filtration on Sephadex G-50 of water-soluble products formed during mild acid hydrolysis of the LPS (Fig. 2). The shape of the elution curve is indicative of a high content of R-form LPS in the native preparation, which is typical of conditionally pathogenic bacteria.

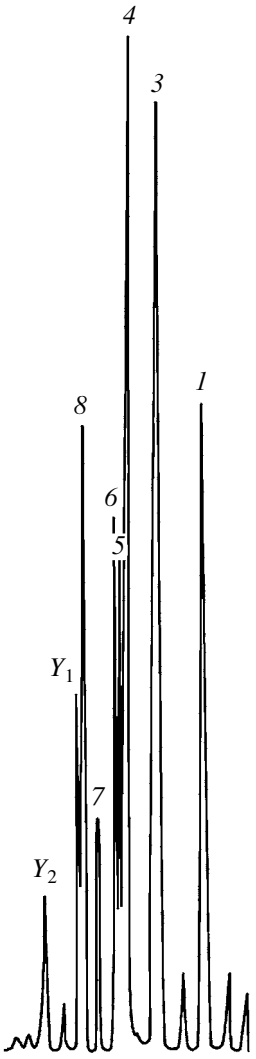

Fig. 1. Fatty acid composition of lipid A from *R. aquatilis*: (*1*) C12:0, (*3*) C14:0, (*4*) C15:0, (*5*) 3-OH-C14:0, (*6*) C16:0, (*7*) C17:0, and (*8*) C18:0. Y_1 and Y_2 are unidentified fatty acids with chain lengths of more than 18.

In the last 6 or 7 years, the development of new methods has facilitated progress in investigation of the

Table 2. Fatty acid composition of the LPS from *R. aquatilis* 1-95

Preparation	Fatty acids (% of the sum of peak areas)								
	$C_{12:0}$	$C_{14:0}$	$C_{15:0}$	3OH-$C_{14:0}$	$C_{16:0}$	$C_{17:0}$	$C_{18:0}$	Y_1	Y_2
LPS	13.8	19.9	21.6	10.3	11.3	3.2	13.3	4.6	2.0

Note: Y_1 and Y_2 are unidentified fatty acids with chain lengths of more than 18.

Table 3. Toxicity and pyrogenicity of the native and modified LPS from *R. aquatilis* 1-95

Preparation, strain		LD_{50}, μg/mouse	Pyrogenicity			
			LPS dose, μg/ml	Temperature change, °C		
				1 h	2 h	3 h
LPS *R. aqutilis*	native	5.0	7.5×10^{-3}	+0.70	+0.54	+0.50
	dephosphorylated	5.0	7.5×10^{-3}	–0.32	–0.33	–0.32
	N,*O*-deacylated	5.0	7.5×10^{-3}	–0.17	–0.28	–0.09
LPS *E. coli* O55:B5		0.14	–	–	–	–
Pyrogenal		–	7.5×10^{-3}	+0.59	+0.69	+0.71

Note: To determine toxicity, the LPSs from *R. aquatilis* 1-95 and *E. coli* were injected into a caudal vein and intraperitoneally, respectively. "–" stands for "not determined".

core oligosaccharide, which is a more variable LPS component than lipid A. It has been shown that the core oligosaccharides from all of the bacterial species studied contain from one to three molecules of KDO, one of which links the lipid A moiety to the polysaccharide. Most core oligosaccharides (but not all, as has been shown recently [17]) contain L- or D-glycero-D-manno-heptoses. In the LPS from *R. aquatilis* 1-95, we revealed only trace amounts of heptose. As can be seen from Table 1, in fraction 2 of the core oligosaccharide, galactose and an unidentified component prevailed, whereas, in fraction 3, arabinose and fucose were predominant. Galactose was earlier revealed in the core oligosaccharides from *Salmonella enterica, S. sonnei*, and *S. flexneri* strains 6, R4, and 4b; *E. coli* strains R1 and R2; *Citrobacter* strains O27, PCM 1487, and O23; *Erwinia carotovora* FERM P-7576; and *Yersinia enterocolitica*, whereas arabinose has been found in the core oligosaccharides from *Proteus mirabilis* R45/1959, *Pseudomonas fluorescens*, and *R. solanacearum* [17]. It is the high content of galactose in the core oligosaccharide of *R. aquatilis* that evidently accounts for the difference between the amounts of galactose revealed in its LPS and O-specific polysaccharide. Heptoses and KDO occurred in fractions 2 and 3 of the core oligosaccharide, respectively. The low content of KDO determined in the *R. aquatilis* core oligosaccharide may be explained by the presence of a C5-substituted KDO residue, which produces a weak reaction with thiobarbituric acid.

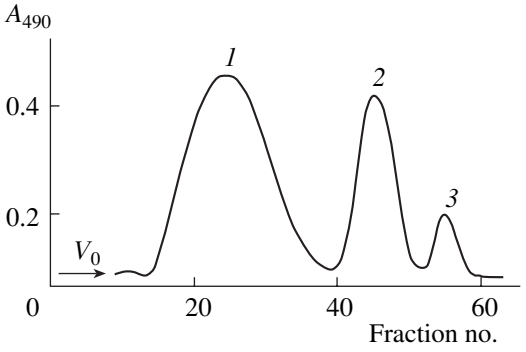

Fig. 2. Elution profile from a Sephadex G-50 column of a degraded LPS from *R. Aquatilis*: (*1*) the O-specific polysaccharide fraction; (*2, 3*) fractions of the core oligosaccharide. V_0 is the dead volume of the column.

The O-PS is the most variable moiety of the LPS molecule. Its structure varied in the course of evolution to deceive the immune system of the host and, thus, protect the bacteria.

The GLC analysis of monosaccarides in the form of polyol acetates obtained after complete acid hydrolysis of the O-PS revealed the presence of galactose and fucose in a ratio of 2 : 1. The GLC analysis of partly methylated polyol acetates revealed 4-O-methylfucopyranose, 2,3,4,6-tetra-O-methylgalactopyranose, and 2,5,6-tri-O-methylgalactofuranose. These results indicate that the O-PS is a branched polysaccharide containing a terminal residue of galactopyranose in its side chain, a 2,3-substituted fucopyranose residue at the point of branching, and a 3-substituted galactose residue in furanose form.

The determination of absolute configurations of the acetylated glycosides with the use of optically active octanol revealed that both monosaccharides had the D configuration.

The ^{13}C-NMR spectrum of the O-PS displayed the signals of 3 anomeric carbon atoms at 96.8–110.3 ppm, 1 methyl group (C6) of a fucose residue at 16.4 ppm, 2 unsubstituted hydroxymethyl groups (C6 Gal) at 62.2–64.2 ppm, and 12 remaining carbon atoms of monosaccharide cycles in the region of 67.9–85.1 ppm (Fig. 3, Table 4). Accordingly, the ^{1}H-NMR spectrum exhibited the signals of three anomeric protons of Fuc at 5.25 ppm, Gal*p* at 5.11 ppm, and Gal*f* at 5.23 ppm; one CH_3-C group (H6 Fuc) at 1.24 ppm; and the remaining sugar protons at 3.68–4.25 ppm (Fig. 4, Table 4).

Thus, the O-PS from *R. aquatilis* 1-95 is strictly regular and is built from repeating branched trisaccharide units consisting of one D-Fuc residue and two D-Gal

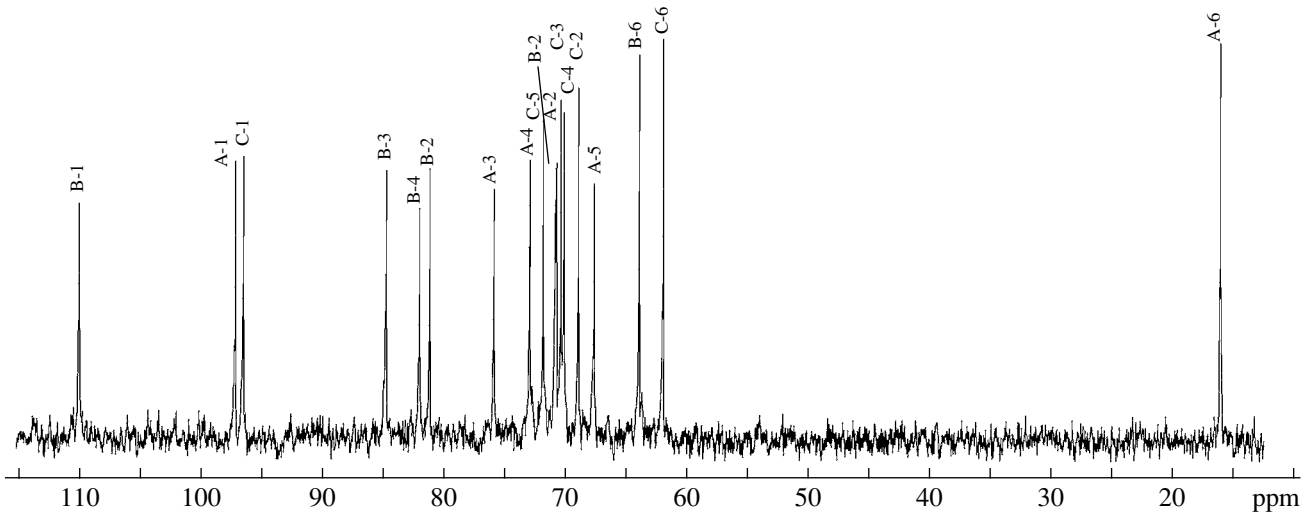

Fig. 3. ^{13}C NMR spectrum of the O-PS from *R. aquatilis.*

residues, one of which, galactopyranose, makes up the side chain. The residue of D-fucopyranose is located at the point of branching and is substituted at positions 2 and 3. The residue of D-galactofuranose is substituted at position 3.

Monosaccharides were also identified with the use of the coupling constant of the spin–spin interaction ($^3J_{H, H}$, H). Based on the characteristic chemical shifts for H-1, C-1, and C-5 (Table 4) and the presence of H-1,H-2 cross-peaks and absence of H-1,H-5 cross-peaks in the NOESY spectrum, it was inferred that the Fuc*p* and Gal*p* residues were connected by an α linkage. The β linkage of the Gal*f* residue was indicated by the chemical shift for C-1 (δ 110.3), since the C-1 atom of the α-Gal*f* residue should have given a signal at about δ 103.

The substitution positions determined in the repeating unit were also confirmed by the results of monosaccharide glycosylation (Table 4). The chemical shifts for the C-2–C-6 atoms in the Gal*p* residue were close to that of an unsubstituted residue of α-galactopyranose. This fact is consistent with the terminal position of this monosaccharide as determined by the methylation method. The sequence of the monosaccharide residues in the repeating unit was determined from the correlation between anomeric protons and protons of the bound carbon atom in the NOESY spectrum. The structure of the repeating unit was described by us in detail elsewhere [18].

Thus, the O-specific polysaccharide from *R. aquatilis* 1-95 is built from repeating branched trisaccharide units with the following structure:

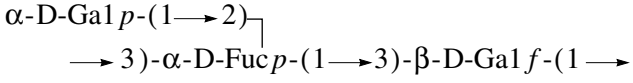

Table 4. Data of 500-MHz ^{1}H NMR and 125-MHz ^{13}C NMR (δ, ppm)

Monosaccharide residue	H-1	H-2	H-3	H-4	H-5	H-6a,6b
→3)-α-D-Fuc*p*-(1→ 2 ↑	5.25	4.10	4.08	3.92	4.25	1.24
→3)-α-D-Gal*f*-(1→	5.23	4.25	4.13	4.22	3.95	3.68
α-D-Gal*p*-(1→	5.11	3.84	3.85	4.01	4.12	3.76
	C-1	C-2	C-3	C-4	C-5	C-6
→3)-α-D-Fuc*p*-(1→ 2 ↑	97.4	71.0	76.2	73.2	67.9	16.4
→3)-α-D-Gal*f*-(1→	110.3	81.4	85.1	82.3	71.1	64.2
α-D-Gal*p*-(1→	96.8	69.2	70.6	70.4	72.1	62.2

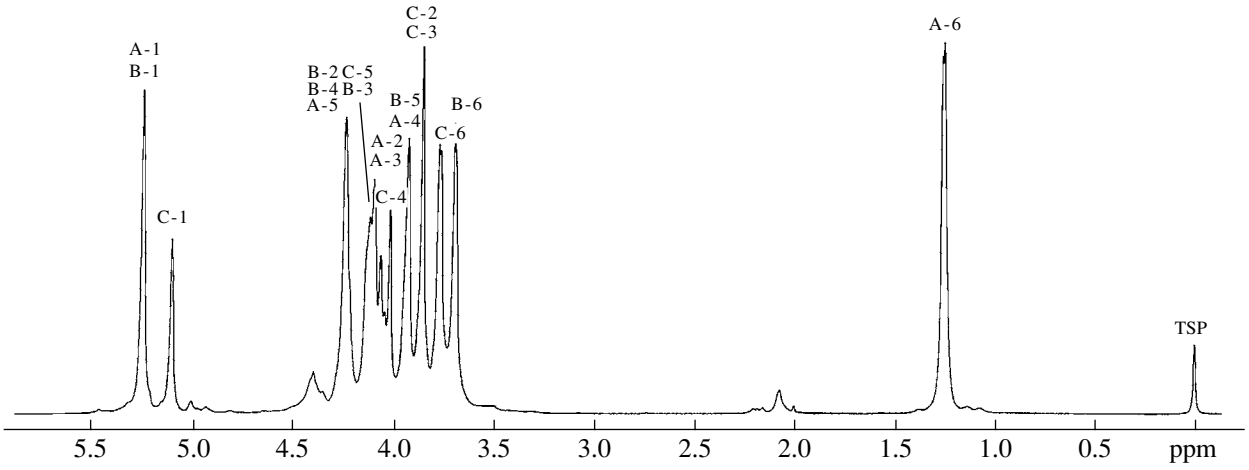

Fig. 4. ^{1}H-NMR spectrum of the O-PS from *R. aquatilis.*

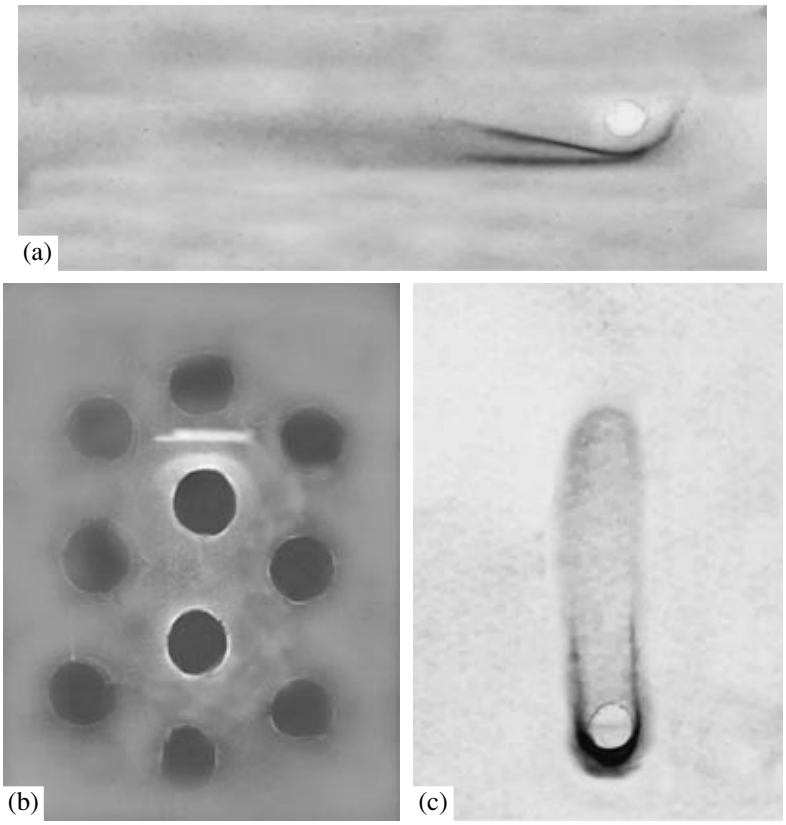

Fig. 5. (a) Immunoelectrophoresis, (b) double diffusion in agar, and (c) rocket immunoelectrophoresis of the LPS from *R. aquatilis* homologous antiserum.

The LPS is the main thermostable antigen of the microbial cells, and its serological specificity is determined by the O-PS structure. Double immunodiffusion in agar gel, immunoelectrophoresis, and rocket immunoelectrophoresis (Figs. 5b, 5a, and 5c, respectively) showed that the *R. aquatilis* 1-95 LPS exhibits serological activity in a homologous system.

Thus, the native preparation of the *R. aquatilis* 1-95 LPS was shown to contain S and R forms of molecules, which is typical of most S forms of gram-negative bacteria. The LPS under study was similar to LPSs from other representatives of the family *Enterobacteriaceae* in relation to the composition of fatty acids. The fraction of core oligosaccharide contained galactose,

fucose, arabinose, and an unidentified component. The O-PS consisted of repeating trisaccharide units composed of fucopyranose and two residues of galactose (in furanose and pyranose forms), of which one was the terminal monosaccharide. The LPS exhibited serological activity; it was less toxic than the LPS from *E. coli* 055:B5 (Sigma, United States) and less pyrogenic than the Pyrogenal formulation.

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