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EXPERIMENTAL ARTICLES

# *Rahnella aquatilis* 95U003 Lipopolysaccharide

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**Abstract**—The lipopolysaccharide of a new species of *Enterobacteriaceae, Rahnella aquatilis* 95U003, was isolated and investigated. The structural components of the lipopolysaccharide molecule, lipid A, core oligosaccharide, and O-specific polysaccharide, were isolated by mild acidic hydrolysis. In lipid A, 3-hydroxytetradecanoic (64.3%) and tetradecanoic (22.3%) acids were found to be predominant fatty acids. In fractions 1 and 2 of the core oligosaccharides, galactose (36.6 and 43.6%), mannose (35.5 and 23.5%), and glucose (42.1 and 25.3%) were shown to be the major monosaccharides. The O-specific polysaccharide consisted of regularly repeating hexasaccharide units of the following structure:

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}p^{I}\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Man}p^{I}\text{-}(1 \rightarrow 2)\text{-}\alpha\text{-D-Man}p^{II}\text{-}(1 \rightarrow$$
$$\uparrow 2 \text{ (of Man}p^{II}) \leftarrow 1$$
$$\beta\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\alpha\text{-D-Gal}p^{II}\text{-}(1 \rightarrow 4)\text{-}\alpha\text{-D-Glc}pA$$

*Key words*: lipopolysaccharide, *Rahnella aquatilis*, O-specific polysaccharide composition and structure

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Microorganisms of the family *Enterobacteriaceae* are an indispensable part of the biosphere. Their polybiotrophy and adaptive flexibility have resulted in their high abundance in various abiotic environmental media, as well as in flora and fauna. The activity of these bacteria affects diverse aspects of human life. The great variety of their biological properties complicates the diagnostics of the family representatives. The novel (newly described) and poorly studied species present most difficulties due to lack of data on their bioecotopes and circulation areas and biological properties and insufficient development of isolation and identification techniques for these microorganisms. However, the rapidly developing traditional methods and fundamentally new diagnostic techniques resulted in the recent discovery of a number of new enterobacterial species, including *Rahnella aquatilis* [1, 2]. These microorganisms were originally isolated from environmental and clinical samples from patients and healthy volunteers in European countries (France, Belgium, and the Czech Republic) with geographical and climatic conditions, flora, and fauna similar to those of Ukraine. Out of the more than 100 microbial species identified as members of the family *Enterobacteriaceae* by the end of 1995, only 20 have attracted the attention of microbiologists; the rest, including *Rahnella aquatilis*, practically have not been studied. In addition to in open water, they have been isolated from soil (most often from the rhizosphere of cereals such as wheat, corn, and rice), as well as clinical samples. *R. aquatilis* is a heterogeneous species with many unsolved taxonomic problems. The composition and structure of lipopolysaccharides (LPSs), the major surface antigens of gram-negative bacteria, are acknowledged chemotaxonomic criteria used for their systematization.

The aim of the present work was to isolate lipopolysaccharide of *R. aquatilis* 95U003; chemically characterize its structural components, that is, lipid A, core oligosaccharide, and O-specific polysaccharide; determine the structure of the latter; and study the serological activity of LPS.

## MATERIALS AND METHODS

The subject of the study was *Rahnella aquatilis* LEAMD 95U003 isolated from the feces of a clinically healthy individual. The culture was isolated and studied by Dr. S. Pokhil. The strain was obtained from the collection of the Laboratory of Experimental Applied

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A culture of *R. aquatilis* was grown in matrasses on nutrient agar at 28°C for 24 h. The cells were washed off with physiological saline, precipitated by centrifugation at 5000 *g* for 30 min, and dried with acetone and ether. Lipopolysaccharides were isolated by the standard water–phenol extraction procedure at 65–68°C [3] followed by removal of nucleic acids by precipitation with trichloroacetic acid and ultracentrifugation at 104 000 *g* for 4 h. LPS preparations free of nucleic acids were lyophilized.

LPS was cleaved by 3% acetic acid (100°C, 6 h), lipid A was precipitated by ultracentrifugation (25 000 *g*, 40 min), and the supernatant, concentrated to the volume of 10 ml, was fractionated on a Sephadex G-50 column (70 ± 3 cm) in pyridine–acetate buffer, pH 4.5. Finally, the fractions of O-specific polysaccharide (OPS) and core oligosaccharide (core OS) were obtained.

Nucleic acids were determined according to Spirin [4]; carbohydrates, with phenol and sulfuric acid [5]; protein, by the Lowry method [6]; heptoses, with cysteine and sulfuric acid [7]; and 2-keto-3-deoxyoktanoic acid (KDO), with thiobarbituric acid [8].

Amino acids and amino sugars were quantified after hydrolysis in 6 N HCl for 20 h at 100°C on a KLA-5 amino acids sequence analyzer (Hitachi).

Neutral monosaccharides were identified after hydrolysis in 2 N HCl (105°C, 5 h) or in 2 M $CF_3CO_2H$ (120°C, 2 h). Alditol acetates were analyzed on an Agilent 6890N/5973 inert chromato–mass spectrometry system, DB-225 mS 30 ± 0.25 mm, 0.25-μm column, in a 1-ml/min helium flow. The temperatures of the evaporator, interface, and thermostat were 250, 280, and 220°C, respectively (isothermal regime). The samples were injected into a carrier flow at a ratio of 1 : 100. An Ultra 2 capillary column on a Hewlett-Packard 5880 instrument was also used, with a temperature gradient from 180 (1 min) to 290°C at 10°C/min [9, 10]. Identification of monosaccharides was carried out by comparing the alditol acetate retention times of the samples and the standards and using the ChemStation database.

The absolute configurations were determined by GLC of acetylated glycosides with (S)-2-octanol using a Hewlett-Packard 5880 instrument at 230°C (20 min) [11].

Methylation of the polysaccharide was carried out by $CH_3I$ in dimethyl sulfoxide in the presence of methylsulfonyl methanide [12]. The methylated polysaccharide was hydrolyzed in 2 M $CF_3CO_2H$ (100°C, 2 h). Partially methylated monosaccharides were reduced with $NaBH^4$, acetylated, and analyzed by GLC–MS on a Hewlett-Packard 5880 instrument equipped with an HP-5ms column using a temperature gradient from 150 (3 min) to 320°C at 5°C/min.

To study the Smith decomposition, 30 mg of OPS was oxidized with 0.1 M $NaIO_4$ water solution for 48 h at 20°C in the dark; then, after the addition of excess ethylene glycol, reduction with $NaBH_4$, and desalting on a TSK HW-40 (S) gel column (80 ± 1.6 cm) in 1% acetic acid at 100°C for 2 h, the product was fractionated by liquid chromatography on a TSK HW-40 (S) gel column (80 ± 1.6 cm) in 1% acetic acid.

The NMR spectra of $^1H$ and $^{13}C$ were recorded with a Bruker DRX-500 spectrometer for solutions in $D_2O$ at 27°C. The chemical shifts were recorded with sodium 3-trimethylsilylpropanoate-$d_4$ ($\delta_H$ 0.00) as an internal standard and acetone ($\delta_C$ 31.45) as an external standard. Mixing times of 200 and 150 ms were used in total correlation spectroscopy (TOCSY) and Overhauser nuclear effect (NOESY) experiments, respectively.

Lipopolysaccharides were hydrolyzed in acetyl chloride (1.5% methanol solution, 100°C, 4 h), and methyl ethers of fatty acids were analyzed with a computerized Agilent 6890N/5973 inert system (United States).

Antisera to the heated (100°C, 2.5 h) *R. aquatilis* culture were obtained by four intravenous immunizations of rabbits (2.0–2.5 kg) with increasing doses of bacterial suspensions ($2 \times 10^6$ to $5 \times 10^7$ cells/ml) at 7-day intervals. The animals were exsanguinated on the 7th day after the last injection. Double diffusion in agar was carried out as described by Ouchterlony in 1% agarose gel in physiological saline with 2% polyethylene glycol 6000 [13]. Immunoelectrophoresis in 1% agarose gel was performed in 0.05 M veronal–medinal buffer solution, pH 8.6, at 80–95 V (12 mA). After electrophoresis, the native antiserum was introduced into each pit and the gel was left overnight at 37°C for precipitation to develop [14].

## RESULTS AND DISCUSSION

The LPS from the strain under study, *R. aquatilis* 95U003, was isolated in amounts of 15.8% of the cell dry weight which is higher than in some *Enterobacteriaceae* species like *Escherichia coli*, yet comparable to that of the new enterobacterial species, such as *R. aquatilis* (5.2–19.8%) and *Pragia fontium* (9.0–22.0%) [15, 16].

The water–phenol procedure is a well-known technique for isolating both LPSs and nucleic acids; the latter are extracted to the phenol layer of the mixture, while LPSs are extracted to the aqueous phase. This technique may cause increased content of nucleic acid as admixtures in LPS preparations. We solved the problem efficiently by removing nucleic acids in several cycles of ultracentrifugation and precipitation of insoluble complexes with trichloroacetic acid. In the purified preparation of *R. aquatilis* 95U003 LPS, relatively high carbohydrate content (64.0%), together with insignificant amounts of protein (0.8%) and nucleic acids (0.4%), was found.

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

| Strain | Structural component | Monosaccharide (% to the total peak area) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Rha | Fuc | Rib | Ara | Man | Gal | Glc |
| 95U003 | LPS | 21.3 | – | 12.0 | 3.0 | 40.1 | 15.1 | 8.4 |
| | O-PS | 2.5 | – | – | 3.4 | 31.3 | 36.6 | 26.2 |
| | core OS 1 | 3.4 | 0.5 | – | – | 35.4 | 36.6 | 24.1 |
| | core OS 2 | 1.2 | – | 1.1 | 5.4 | 23.5 | 43.5 | 25.3 |

**Table 2.** Amino acid composition (% to total dry weight) of the LPS of *R. aquatilis* LEAMD 95U003

| Amino acid | % to total dry weight | Amino acid | % to total dry weight |
|---|---|---|---|
| Aspartic acid | – | Isoleucine | – |
| Threonine | – | Leucine | 0.2 |
| Serine | 0.2 | Tyrosine | – |
| Glutamic acid | 0.5 | Phenylalanine | – |
| Glycine | 0.4 | Histidine | 0.5 |
| Alanine | 0.4 | Ornithine | 0.2 |
| Valine | – | Lysine | – |
| Methionine | – | Arginine | – |
| Diaminopimelic acid | – | Cysteine | – |

Note: "–" stands for unidentified.

A study of the monosaccharide composition of the LPS (Table 1) revealed mannose (40.1%), rhamnose (21.3%), and galactose (15.1%) as major monosaccharides. Ribose (12.0%), glucose (8.4%), and arabinose (3.0%) were also present. Among hexoseamines, only glucosamine (0.5%) was detected.

In the LPS of the strain under study, core oligosaccharide components were revealed, which are rarely found in other natural biopolymers, i.e., L-glycero-D-mannoheptose (2.2%) and 2-keto-3-deoxyoktanoic acid (KDO) (0.1%). KDO is the only structural component present in all bacterial LPSs regardless of their systematic position. Bacteria with deficient KDO synthesis apparatus are not viable, which indicates that KDO is absolutely necessary for the structural and functional integrity of a cell.

Analysis of amino acid composition of the *R. aquatilis* 95U003 LPS (Table 2) demonstrated that they were present in insignificant amounts, 0.2–0.5%, with glutamic acid, histidine, glycine, and alanine as predominant amino acids.

LPS is a complex molecule in which the carbohydrate moiety is coupled to lipid A by the KDO glycoside bond. The bond is highly sensitive to acids, and thus may be selectively cleaved by mild acidic hydrolysis. Therefore, LPS may be easily fractionated into the water-insoluble lipid A and water-soluble carbohydrate matter (so-called degraded LPS). The LPS of *R. aquatilis* 95U003 required more severe conditions of hydrolysis compared to the LPSs of other gram-negative bacteria evidencing high stability of the LPS ketoside bond. After degradation, the lipid A yield was 5.2%.

Lipid A is the most evolutionary conservative part of the bacterial LPS. Lipids A of various bacteria are known to differ by fatty acid composition, which is a relatively stable characteristic and thus may be used as a chemotaxonomic criterion to establish the phylogenetic relation between microorganisms, as well as to

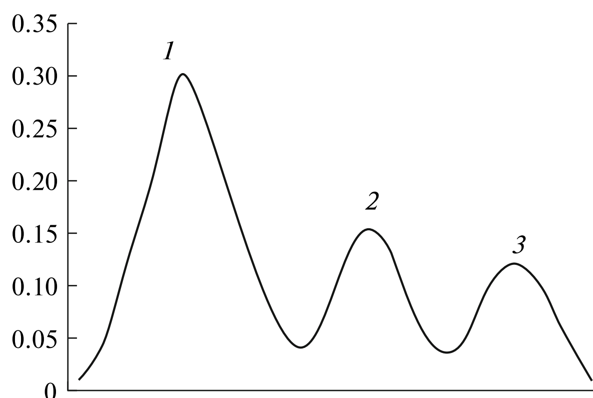


**Fig. 1.** Elution profile of the carbohydrate moiety of the degraded LPS molecule of *R. aquatilis* 95U003 on Sephadex G50: OPS fraction (*1*), core oligosaccharide fractions (*2, 3*).

solve some taxonomic questions. Hydroxylated acids binding directly to the carbon chain of lipid A are of diagnostic value. Analysis of the chromato–mass profiles of fatty acids methyl ethers revealed the presence of fatty acids with a $C_{12}$ to $C_{16}$ carbon chain. The dominant ones were 3-hydroxytetradecanoic (64.3%) and tetradecanoic (22.3%) acids. Hexadecanoic (7.2%) and dodecanoic (6.2%) acids were also detected in lipid A of *R. aquatilis* 95U003. The details of fatty acid composition are known to be species-specific. For the family *Enterobacteriaceae*, the presence of one hydro xyacid—namely, 3-hydroxytetradecanoic acid—is characteristic, which may acylate both amino and hydroxyl groups of glucosamine. Therefore, the data obtained verify additionally that *R. aquatilis* 95U003 was classified among the enterobacteria correctly.

Other structural components, that is, O-specific polysaccharide (OPS) (fraction 1) and the core oligosaccharide (core OS) (fractions 2 and 3), were isolated by gel filtration on a Sephadex G-50 column from the carbohydrate part of degraded LPS. Carbohydrate elution profiles (Fig. 1) indicate that the initial LPS and, thus, the bacterial population under study contained S and R forms of the LPS with predomination of the high molecular weight OPS fraction and presence of some low-molecular fractions of the core oligosaccharide.

LPS heterogeneity, that is, the presence of both S and R forms, was also confirmed by SDS-PAGE (Fig. 2). Detection of both slowly and rapidly migrating fractions correlates to the data that were obtained in gel filtration experiments and is evidence that the rapidly migrating fractions corresponded to unsubstituted core OS and the slow fractions corresponded to the core OS substituted with O-specific polysaccharide chains. The heterogeneity of slowly migrating components may be ascribed to the presence of several polysaccharide chains in the LPS differing by molecular weight due to the presence of the molecules of a wide spectrum of lengths.

In both fractions of the core OS (1 and 2, respectively), galactose (36.6 and 43.6%), mannose (35.5 and 23.5%), and glucose (24.1 and 25.3%) were found to be dominating. Insignificant amounts of arabinose (core OS 2), rhamnose (core OS 1 and 2), ribose (core OS 2), and fucose (core OS 1) were detected.

A study of the OPS monosaccharide composition demonstrated that the main neutral monosaccharides were galactose, mannose, and glucose in a ratio of 1 : 1 : 0.6. Anion-exchange chromatography in a carbohydrate analyzer revealed the presence of glucuronic acid. Analysis of the monosaccharide absolute configuration revealed that they all were in D-configuration. Methylation analysis, including GLC of partially methylated alditol acetates, revealed 2,3,4,6-tetra-O-methylglucopyranose, 3,4,6-tri-O-methylhexapyranose, 2,4,6-tri-O-methylhexapyranose, and 4,6-di-O-metylpyranose. Besides these monosaccharides, reduction of the methylated polysaccharide carboxyl revealed 2,3-di-O-methylhexose, which apparently originated from glucuronic acid substituted at position 4. The data indicate that the OPS is a branched one, the side chain terminating with glucose.

The $^{13}$C NMR spectrum of the OPS (Fig. 3) contained signals of six anomeric atoms at 96.4–105.1 ppm, five hydroxymethyl groups (C-6 Glc, Man, and Gal) at 62.2–62.7 ppm, one carboxyl group of GlcA residue at 175.6 ppm, and signals of other carbon atoms of the monosaccharide rings at 66.2–80.6 ppm. Correspondingly, $^{1}$H NMR of the OPS contained signals of six anomeric protons at 4.61–5.46 ppm and of the other sugar protons at 3.03–4.23 ppm. The absence of signals at 83–88 ppm in the $^{13}$C spectrum evidences that all sugar residues are in pyranose form [17].

Spin systems of all monosaccharide residues were assigned according to combined 2D COSY, TOCSY, and NOESY spectra (Table 4).

The $^{13}$C NMR spectrum was deciphered with a two-dimensional $^{1}$H,$^{13}$C heteronuclear HSQC experiment (Table 5). Signals of C-2 in Man$^{II}$ at 80.7 ppm;

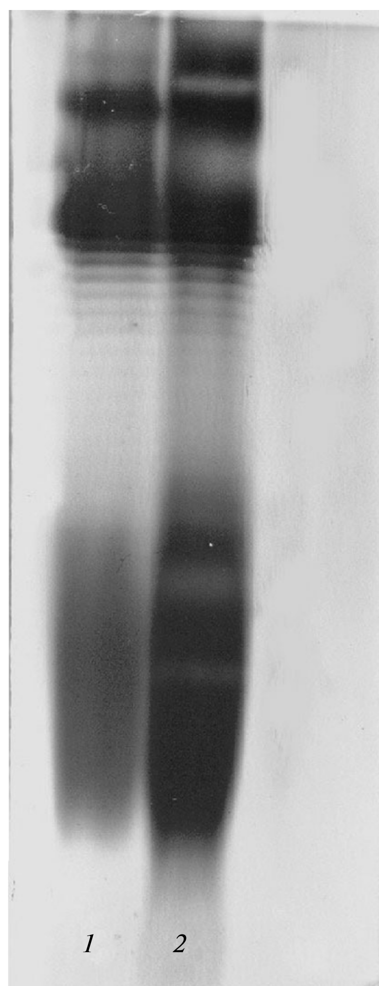


**Fig. 2.** Electrophoresis of the LPS of *R. aquatilis* 95U003 (*1*) and 95U004 (*2*).

C-2 and C-3 in $Man^{I}$ at 77.6 and 79.3 ppm, respectively; C-3 in $Gal^{I}$ and $Gal^{II}$ at 78.3 and 80.6 ppm, respectively; and C-4 GlcA at 78.6 ppm appeared significantly shifted toward a weak field in comparison to their position in the spectra of the corresponding unsubstituted hexapyranosides at 70–73 ppm [17]. These shifts, caused by the glycosylation α effect, allowed determination of the substituted positions in the monosaccharides and correlated well with the methylation data. In the NOESY spectrum of the OPS, interunit correlation peaks between the following transglycoside protons were observed: Glc H-1/$Gal^{II}$ H-3 at 4.69/3.97 ppm, $Gal^{II}$ H-1/GlcA H-4 at 5.46/3.81 ppm, GlcA H-1/$Man^{I}$ H-2 at 5.42/4.30 ppm, $Man^{I}$ H-1/$Man^{II}$ H-2 at 5.28/4.04 ppm, $Man^{II}$ H-1/$Gal^{I}$ H-3 at 5.23/3.76 ppm, and $Gal^{I}$ H-1/$Man^{I}$ H-3 at 4.61/4.18 ppm, respectively. The following cross-peaks were also observed: $Man^{I}$ H-1/GlcA H-5 and $Man^{II}$ H-1/$Man^{I}$ H-5 at 5.28/4.27 and 5.23/3.79 ppm, confirming the presence of fragments α(1 ⟶ 2)-α-$Man^{I}$ and α-$Man^{I}$-(1 ⟶ 2)-α-$Man^{II}$ in the OPS. The data are in agreement with the branched nature of

**Table 3.** Fatty acid composition of the lipid A of the LPS of *R. aquatilis* 95U003

| Strain | Fatty acids (% of total peak area) | | | | | | |
|---|---|---|---|---|---|---|---|
| | C12 : 0 | C14 : 0 | C15 : 0 | 3 OH–C14 : 0 | C16 : 0 | C18 : 0 | C20 : 0 |
| 95U003 | 6.20 | 22.30 | – | 64.30 | 7.20 | – | – |

**Fig. 3.** $^{13}$C NMR spectrum of the *R. aquatilis* 95U003 O-specific polysaccharide.

β-D-Gal*p*$^{II}$-(1 → 3)-α-D-Man*p*$^{I}$-(1 → 2)Gro-al **1**

β-D-Gal*p*$^{II}$-(1 → 3)-α-D-Man*p*$^{I}$-(1 → O ... $CH_2OH$ **2**

α-D-Gal*p*$^{I}$-(1 → 3)-EryA **3**

α-D-Gal*p*$^{I}$-(1 → O ... **4**

**Fig. 4.** Structure of the oligosaccharides of *R. aquatilis* 95U003 obtained upon OPS Smith degradation.

the polysaccharide and the substituted positions defined from methylation and $^{13}$C NMR spectrum data (see above). Therefore, the O-repeating unit of *R. aquatilis* 95U003 OPS is of the following structure:

→ 3)-β-D-Gal*p*$^{I}$-(1 → 3)-α-D-Man*p*$^{I}$-(1 → 2)-α-D-Man*p*$^{II}$-(1 →
2
↑
1
β-D-Glc*p*-(1 → 3)-α-D-Gal*p*$^{II}$-(1 → 4)-α-D-Glc*p*A

To confirm the established structure, the OPS was subjected to Smith degradation. The oxidation products after mild acidic hydrolysis were fractionated liquid chromatography on a TSK HW-40 column. The resulting oligosaccharide fractions were analyzed with NMR spectroscopy as was described for the OPS ($^{1}$H and $^{13}$C NMR data on chemical shifts are presented in Tables 4 and 5 and the oli-

**Table 4.** 500-MHz $^1$H NMR spectrum of the *R. aquatilis* 95U003 O-polysaccharide and oligosaccharides obtained upon Smith degradation of the *R. aquatilis* 95U003 OPS (chemical shifts in ppm)

| Moiety | 1 | 2(2a/2b) | 3(3a/3b) | 4(4a/4b) | 5 | 6(6a) | 6b |
|---|---|---|---|---|---|---|---|
| OPS | | | | | | | |
| → 3)-α-D-Gal$p^{II}$-(1 → | 5.46 | 3.99 | 3.97 | 4.23 | 3.94 | 3.70 | 3.70 |
| → 2,3)-α-D-Man$p^{I}$-(1 → | 5.28 | 4.30 | 4.18 | 3.89 | 3.79 | 3.91 | 3.80 |
| → 4)-α-D-Glc*p*A-(1 → | 5.24 | 3.63 | 4.00 | 3.81 | 4.27 | | |
| → 2)-α-D-Man$p^{II}$-(1 → | 5.23 | 4.04 | 4.01 | 3.71 | 3.88 | 3.87 | 3.76 |
| β-D-Glc*p*-(1 → | 4.69 | 3.37 | 3.53 | 3.43 | 3.46 | 3.89 | 3.74 |
| → 3)-β-D-Gal$p^{I}$-(1 → | 4.61 | 3.67 | 3.76 | 4.14 | 3.68 | 3.87 | 3.78 |
| Oligosaccharide **1** | | | | | | | |
| → 3)-β-D-Gal$p^{I}$ | 4.54 | 3.61 | 3.69 | 3.93 | 3.71 | 3.75 | |
| → 2,3)-a-D-Man$p^{I}$ | 5.14 | 4.21 | 4.11 | 3.82 | 3.91 | 3.75 | 3.89 |
| Gro | 5.14 | 3.93 | 3.86/3.77 | | | | |
| Oligosaccharide **2** | | | | | | | |
| → 3)-β-D-Gal$p^{I}$ | 4.54 | 3.61 | 3.69 | 3.93 | 3.71 | 3.75 | |
| → 2,3)-α-D-Man$p^{I}$ | 5.12 | 4.22 | 4.06 | 3.82 | 3.91 | 3.75 | 3.89 |
| → 2)-α-D-Man$p^{II}$* | 3.71 | 3.86/3.77 | 5.13 | 3.63/3.72 | 4.30 | 3.81/4.03 | |
| Oligosaccharide **3** | | | | | | | |
| α-D-Gal$p^{II}$-(1 → | 5.19 | 3.80 | 3.89 | 4.01 | 4.09 | 3.73 | |
| EryA | | 4.32 | 4.02 | 3.73/3.79 | | | |
| Oligosaccharide **4** | | | | | | | |
| α-D-Gal$p^{II}$-(1 → | 5.12 | 3.86 | 3.92 | 3.99 | 4.17 | 3.75 | |
| EryA 1,4-lactone | | 4.68 | 4.80 | 4.45/4.54 | | | |

* Dioxolane derivative from the oxidized Man$p^{II}$ residue.

**Table 5.** Data on 125-MHz $^{13}$C NMR spectrum of the *R. aquatilis* 95U003 O-polysaccharide and oligosaccharides obtained upon Smith degradation of the *R. aquatilis* 95U003 OPS (chemical shifts in ppm)

| Moiety | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| OPS | | | | | | |
| → 3)-α-D-Gal$p^{II}$-(1 → | 100.3 | 69.0 | 80.6 | 70.3 | 72.1 | 62.2 |
| → 2,3)-α-D-Man$p^{I}$-(1 → | 102.2 | 77.6 | 79.3 | 67.2 | 74.9 | 62.7 |
| → 4)-α-D-Glc*p*A-(1 → | 101.4 | 72.9 | 74.8 | 78.6 | 73.3 | 175.6 |
| → 2)-α-D-Man$p^{II}$-(1 → | 96.4 | 80.7 | 71.5 | 68.6 | 74.3 | 62.6 |
| β-D-Glc*p*-(1 → | 105.1 | 75.0 | 77.2 | 71.1 | 77.3 | 62.2 |
| → 3)-β-D-Gal$p^{I}$-(1 → | 102.5 | 70.9 | 78.3 | 66.2 | 76.6 | 62.7 |
| Oligosaccharide **1** | | | | | | |
| → 3)-β-D-Gal$p^{I}$ | 102.5 | 72.3 | 74.2 | 70.1 | 76.7 | 62.4 |
| → 2,3)-α-D-Man$p^{I}$ | 100.3 | 69.1 | 78.8 | 66.6 | 74.4 | 60.9 |
| Gro | 90.5 | 77.8 | 62.6 | | | |
| Oligosaccharide **2** | | | | | | |
| → 3)-β-D-Gal$p^{I}$ | 102.5 | 72.3 | 74.2 | 70.1 | 76.7 | 62.4 |
| → 2,3)-α-D-Man$p^{I}$ | 100.7 | 69.2 | 78.8 | 66.6 | 74.4 | 60.9 |
| → 2)-α-D-Man$p^{II}$* | 81.2 | 62.6 | 104.2 | 63.4 | 78.7 | 67.9 |
| Oligosaccharide **3** | | | | | | |
| α-D-Gal$p^{II}$-(1 → | 100.5 | 70.1 | 70.8 | 70.6 | 73.9 | 62.6 |
| EryA | n.d. | 74.4 | 82.2 | 61.8 | | |
| Oligosaccharide **4** | | | | | | |
| α-D-Gal$p^{II}$-(1 → | 97.8 | 69.5 | 70.7 | 70.7 | 72.9 | 62.7 |
| EryA 1,4-lactone | n.d | 70.3 | 74.4 | 71.6 | | |

Notes: n.d. – undefined
* Dioxolane derivative from the oxidized Man$p^{II}$ residue.

gosaccharide structures are in Fig. 4). The Smith degradation data confirm the *R. aquatilis* 95U003 OPS structure established by methylation and NMR spectroscopy techniques [18].

The serological specificity of a bacterial cell is known to be determined by the specific composition and structure of its LPS. To study the immunochemical properties of *R. aquatilis* 95U003 LPS, polyclonal O-antisera against heat-killed cultures of a homologous strain and *R. aquatilis* type strain 33071 were used as antibodies. In experiments on double immunodiffusion in agar according to Ouchterloni (Fig. 5a), immunoelectrophoresis (Fig. 5b), and rocket immunoelectrophoresis (Fig. 5c), the LPS of *R. aquatilis* 95U003 reacted with both the homologous antiserum and the antiserum to the *R. aquatilis* type strain 33071, indicating common antigen determinants and thus referring the strain to the same serogroup as the type strain.

Therefore, LPS of *R. aquatilis* 95U003, a representative of a new species of *Enterobacteriaceae*, was isolated. Its structural components, O-specific polysac-

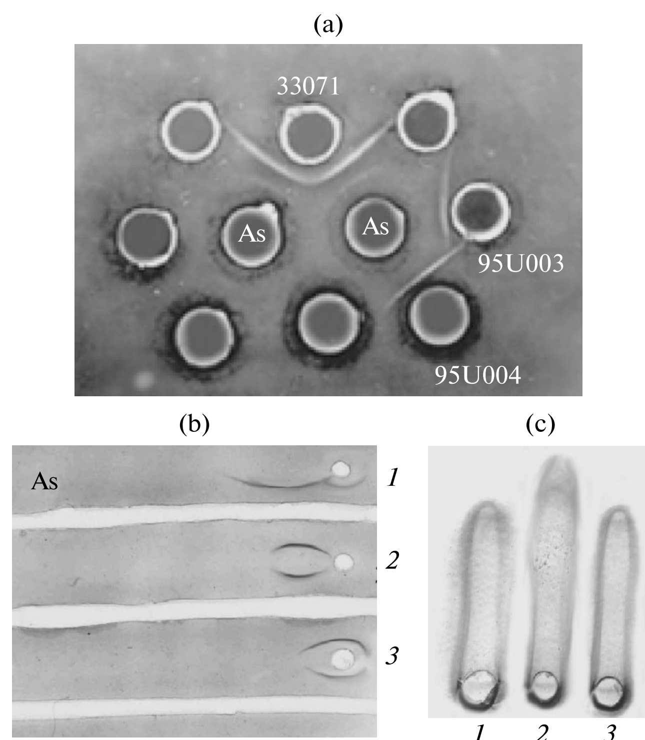


**Fig. 5.** Double immunodiffusion in agar (a), immunoelectrophoresis (b), and rocket immunoelectrophoresis (c) of the *R. aquatilis* type strain 33071 (*1*) and strains 95U003 (*2*) and 95U004 (*3*) with antiserum (As) against *R. aquatilis* 33071.

charide, core oligosaccharide, and lipid A, were separated and identified chemically; the structure of the O-specific polysaccharide was established, and the presence of antigen determinants common to those of the reference strain *R. aquatilis* ATCC 33071 was demonstrated.

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