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EXPERIMENTAL ARTICLES

Characterization of the Lipopolysaccharides of Serogroup II *Azospirillum* strains

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Abstract—Lipopolysaccharides of six *Azospirillum brasilense* strains (SR50, SR80, SR88, SR109, SR111, SR115) and *A. lipoferum* SR 42 isolated from the rhizosphere of cereal plants of Saratov oblast, Russia and referred to serogroup II by serological analysis were studied. In the lipid A fatty acid composition, the lipopolysaccharides under study were similar to those of other *Azospirillum* strains and were characterized by a predominance of 3-hydroxytetradecanoic, 3-hydroxyhexadecanoic, and octadecenoic acids. Monosaccharide analysis of the O-specific polysaccharides (including determination of the absolute configurations, methylation analysis, and one- and two-dimensional NMR spectroscopy) revealed the presence of two types of repeating units in varying ratios. A high degree of serological similarity between the strains under study was shown to result from the presence of repeating units with identical structure of their O antigens.

Keywords: lipopolysaccharides, structure of the O-specific polysaccharide, serological investigation, *Azospirillum*

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Free-living nitrogen-fixing bacteria of the genus *Azospirillum* belong to the *Alphaproteobacteria*; they stimulate growth and development of plants, in particular, cereals and fodder crops [1]. The studies on the structure and properties of the main surface components of *Azospirillum* are of great importance for understanding the mechanisms involved in the formation of bacteria–plant associations, as well as for successful biotechnological application of azospirilla. The complicated mechanism of plant–microbial interactions also includes variability of the polysaccharides and proteins of the cell surface [2].

Similarly to other gram-negative bacteria, *Azospirillum* produce lipopolysaccharides (LPS), which form the outer layer of the outer membrane and are involved in the interactions with various macro- and microorganisms. The amphiphilic LPS molecule consists of a hydrophobic component (lipid A), through which the core oligosaccharide (core) is bound with an O-specific polysaccharide (O-antigen, O-chain, OPS) containing repeating oligosaccharide elements, or units. The S form of the LPS is characterized by the presence of all three parts of the molecule; while in the R form, the carbohydrate part is represented by the core oligosaccharide alone. It should be noted that there is no information on the wild or mutant *Azospirillum* R-phenotypes of LPS without the O-chain.

Antigenic determinants located in the OPS may be used for development of serological classifications (serotyping) of bacteria. Earlier, polyclonal rabbit antibodies (Ab) were obtained against the LPS preparations from a number of *Azospirillum* strains. Based on serological studies of the *Azospirillum* O-antigens, a classification scheme was suggested which included three serogroups for strains of the species *A. brasilense, A. lipoferum,* and *A. irakense* [3, 4]. The chemical basis for the serological cross-reaction between the azospirilla of serogroups I and III is determined by the presence of linear either D- or L-rhamnan oligosaccharide fragments in the main OPS chain [5, 6]. Strains belonging to the serogroup II *Azospirillum* were characterized by the presence of heteropolysaccharide O-antigens and demonstrated a cross-reaction with the antibodies against LPS of the type strain *A. brasilense* Sp7. However, detailed serological analysis of the representatives of serogroup II revealed the heterogeneity of their O-antigenic determinants and indicated the necessity of further typing of bacteria on the basis of the chemical structure of their LPSs.

This paper contains results of structural and serological studies of O-antigens from six *A. brasilense* strains and one strain of *A. lipoferum*, which are serologically close to strain *A. brasilense* SR80, a representative of serogroup II azospirilla, for which the OPS structure had been recently established [7].

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Table 1. Bacterial strains used in the study

Strain	Characteristics
A. brasilense	
SR50	Wheat germs (*Triticum aestivum* L.) Saratovskaya variety 52
SR80	Wheat germs (*T. aestivum* L.) Saratovskaya variety 49
SR88	Wheat germs (*T. durum* Desf.) Khar'kovskaya variety 46
SR109	Maize germs (*Zea mays* L.) Zubovidnaya variety
SR111	Millet germs (*Panicum miliaceum*) Saratovskaya variety 853
SR115	Sorghum germs (*Sorghum bicolor* (L.) Moench) Saratovskoe sakharnoe variety
A. lipoferum	
SR42	Wheat roots (*T. aestivum* L.) Sarrubra variety

MATERIALS AND METHODS

Bacterial strains used in this study (Table 1) were isolated from the rhizosphere of various cereals (Saratov oblast, Russia) and kindly provided by the Collection of Rhizosphere Microorganisms of the Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences (Saratov). Bacteria were cultivated in liquid malate–salt medium with vitamins [8] until the end of the exponential growth phase at 30°C on a shaker. The cells were collected by centrifugation and resuspended in 0.15 M NaCl; capsular material was removed from the cell surface by mechanical stirring for 5 days with replacement of the washing solution each 12 h.

The LPS was extracted from the acetone-dried capsule-free cells with hot 45% aqueous phenol without subsequent layer separation [9]. Protein impurities were precipitated from the LPS solution by addition of 40% CCl_3CO_2H, up to the final pH of 2.7.

The LPS degradation was performed with 2% CH_3CO_2H at 100°C for 4–5 h; the formed lipid A precipitate was separated by centrifugation. The water-soluble fractions were separated by gel chromatography on a Sephadex G-50 column with 0.05 M pyridine–acetate buffer (pH 4.5) as an eluent. The separated products in the eluate were detected using a differential flow refractometer (Knauer, Germany).

Denaturing electrophoresis of the LPS preparations was carried out in 15% PAG [10]. The LPS components were visualized by staining with silver nitrate [11]. Immunochemical studies were performed using polyclonal rabbit Ab (100 μg/mL) against the glutaraldehyde-treated whole cells of *A. brasilense* Sp245, as well as Ab against LPSs from *A. brasilense* Sp7, SR80, and Jm6B2. For immunoblotting [12], the separated components were electrically transferred from the gel onto nitrocellulose membranes with pore size of 0.2 μm (Sigma, United States) in an electric field at a current strength of 200 mA for 1.5 h. Free binding sites were blocked with 1% skimmed milk for 1 h. Immunodetection was carried out by incubating the blots with polyclonal rabbit Ab; goat antirabbit antibodies conjugated with horseradish peroxidase (Sigma, United States) were used for visualization with 3,3'-diaminobenzidine as a substrate. Double radial immunodiffusion of the LPS preparations was carried out by the standard method in 1% agarose gel [13]. The precipitate was stained with Coomassie Brilliant Blue R-250. Enzyme-linked immunosorbent assay (ELISA) was performed in 96-well plates (Medpolimer, Russia). Successive twofold dilutions of the samples in 0.15 M phosphate buffer, pH 7.2 (50 μL) were added into the wells; concentration of the LPS preparation in the first well was 50 μg/mL. Goat antirabbit antibodies conjugated with horseradish peroxidase (Sigma, United States) were used for visualization. Hydrogen peroxide with *o*-phenylenediamine was applied as a substrate reagent. The optical density measurements of the tested samples were carried out at 492 nm using a Multiscan Ascent immune enzyme analyzer (Thermo scientific, Finland). To compare the interactions of LPS preparations with the antibodies, the values of optical density of the reaction products obtained at the maximal LPS concentration were used.

Colorimetric determinations of carbohydrates, 2-keto-3-deoxyoctanoic acid (KDO), and phosphorus in the LPS preparations were carried out by the methods described earlier [8]. The measurements were performed on a Specord 40 spectrophotometer (Analytik Jena AG, Germany).

The composition of fatty acid methyl esters (FAME) was analyzed by gas-liquid chromatography (GLC) on a GC-2010 chromatograph (Shimadzu, Japan) equipped with a DB-5 column (Agilent, United States). Methylation of fatty acids was performed as described earlier [14]. Analysis of monosaccharide composition of the polysaccharides and determination of absolute configurations of neutral sugars were carried out by GLC of acetate polyols [15] and acetylated glycosides with (R)-2-octanol [16], respectively.

Table 2. Chemical composition of the LPSs from the studied azospirilla strains

Components		Strain						
		SR42	SR50	SR80	SR88	SR109	SR111	SR115
% of the LPS mass	Carbohydrates	34.1 ± 2.2	54.9 ± 3.7	29.3 ± 3.1	47.2 ± 5.4	47.8 ± 1.7	48.9 ± 3.1	40.4 ± 2.5
	KDO	1.4 ± 0.1	0.5 ± 0.1	2.7 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	1.6 ± 0.1
	Phosphorus	0.50 ± 0.06	3.0 ± 0.3	0.10 ± 0.03	2.3 ± 0.1	3.4 ± 0.1	0.5 ± 0.1	0.17 ± 0.04
% of the total peak areas FAME	3-OH-$C_{14:0}$	36.0 ± 4.6	37.9 ± 4.9	49.8 ± 1.2	24.6 ± 2.2	34.8 ± 2.0	38.9 ± 4.1	40.0 ± 3.4
	$C_{16:1}$	3.4 ± 0.1	2.7 ± 0.1	Tr	5.6 ± 0.7	3.6 ± 0.8	1.8 ± 0.3	2.8 ± 0.1
	$C_{16:0}$	3.9 ± 0.1	4.4 ± 2.5	5.3 ± 0.4	5.5 ± 0.8	4.4 ± 0.7	3.7 ± 0.5	6.7 ± 0.9
	3-OH-$C_{16:0}$	14.4 ± 2.1	18.3 ± 1.8	23.9 ± 0.7	10.6 ± 3.6	15.6 ± 1.0	20.0 ± 2.1	15.1 ± 1.9
	$C_{18:1}$	36.2 ± 3.9	32.2 ± 5.6	22.3 ± 0.4	49.1 ± 7.3	38.0 ± 4.5	30.9 ± 3.3	33.9 ± 1.0
	$C_{19:0}$	5.5 ± 0.7	4.4 ± 1.3	1.7 ± 0.3	Tr	Tr	4.8 ± 0.6	–

"Tr" signifies that the content of a component was less than 1%; "–" stands for "not detected."

The NMR spectra were recorded on DRX-500 and Avance II 600 spectrometers (Bruker, Germany) in 99.96% solution of D_2O at 30°C with acetone (δ_C 31.45) and 3-trimethylsilyl propanoate-d_4 (δ_H 0.0) as internal standards. The samples were preliminarily twice lyophilized from 99.9% D_2O. Two-dimensional spectra were performed with the aid of the bundled software package (Bruker, Germany); the data were processed using the XWINNMR 2.1 program. Mixing times in the total correlation spectroscopy (TOCSY) and rotating-frame nuclear Overhauser effect correlation spectroscopy (NOESY) spectra were set to 150 and 200 ms respectively.

RESULTS AND DISCUSSION

The LPS extracted from dry biomass of azospirilla by water–phenol mixture contained all the typical components: carbohydrates (29–55%) including heptose and KDO (0.5–2.7%), phosphorus (0.1–3.4%), and 3-hydroxyalkanoic acids (Table 2). The LPS yield reached 5–8% of the acetone-dried biomass.

According to GLC analysis, lipid A contained fatty acids (FA) with 14–19 carbon atoms (Table 2), which had been previously revealed in other strains of *A. brasilense* and *A. lipoferum* [17, 18]. The predominant acids (3-hydroxytetradecanoic, 3-hydroxyhexadecanoic, and octadecenoic) reached 85% of the total identified FAME. Hexadecanoic, hexadecenoic, and nonadecanoic acids were found in minor amounts. Lipids A of all previously studied azospirilla LPSs were characterized by a high content of unsaturated FA, mainly octadecenoic acid [17, 18]. Their level in the LPSs of the strains studied in the present work varied from 32 to 49%. Only in strain *A. brasilense* SR88 did unsaturated FA prevail over 3-hydroxy acids.

The presence of unsaturated FA (which are able to increase membrane fluidity) in lipid A appeared to promote the adaptation of soil bacteria to a wide range of ambient temperatures. Unsaturated FA were revealed in the LPS of soil bacteria *Rhodobacter sphaeroides* capable of nitrogen fixation [19] and in the widespread bacteria of the genus *Pseudomonas* [20, 21]. Enterobacteria *Escherichia coli* and *Salmonella enterica* were shown to accumulate unsaturated FA in their LPSs during cultivation at decreased temperatures (10–15°C) [22, 23].

Electrophoretic analysis of the LPS preparations from the strains under study with subsequent visualization of components by staining with silver nitrate made it possible to identify the predominance of S-forms and lower amounts of R-forms of the LPS molecules (Fig 1a). The only exception was the LPS from *A. brasilense* SR50, which was characterized by heterogeneity of the O-chain lengths and a considerable content of R-forms.

Serological studies revealed interactions of the isolated LPS preparations with the antiserum against the LPS from *A. brasilense* SR80. The ELISA test showed interactions at the level of homologous antigen (Fig. 2), whereas radial immunodiffusion revealed interstrain differences in the number and localization of the precipitation bands (Fig. 3). The immunoblotting test revealed common antigenic determinants in S-forms of LPSs from the studied strains (Fig. 1b), which made it possible to suggest structural similarity of their O-specific polysaccharides. Moreover, the studied LPS preparations showed cross-reactions with the antibodies against the LPSs from strains *A. brasilense* Sp7, Sp245, and Jm6B2; however, intensity of their interactions varied considerably depending on the antigen/antibody combinations (Fig. 2). This fact is of special interest since the model strains

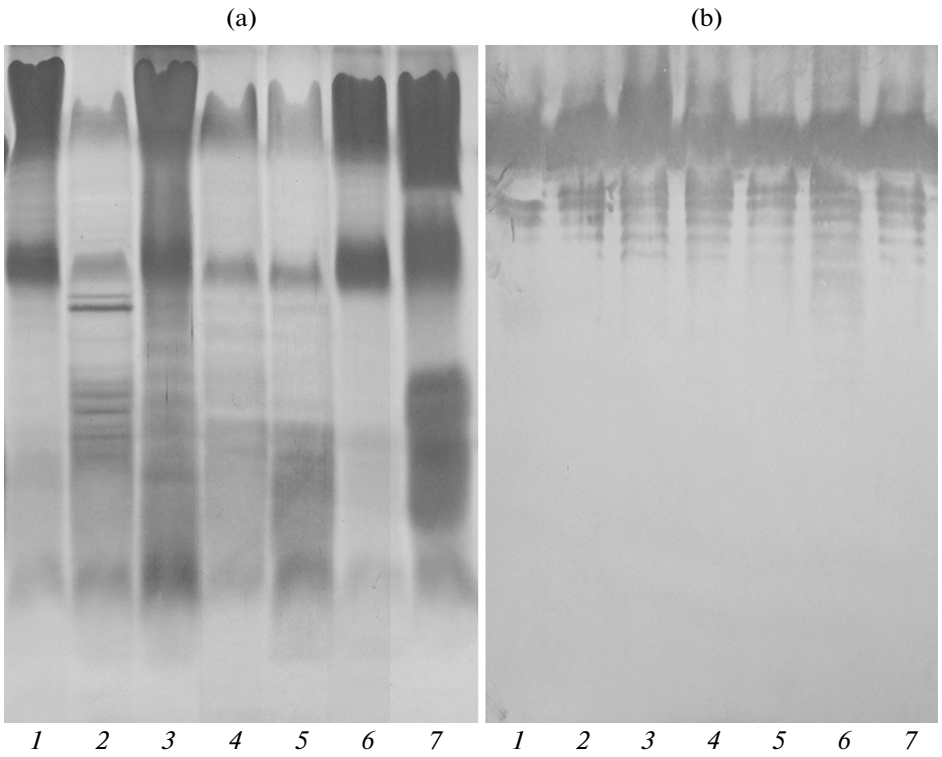

Fig. 1. Electrophoregram of the LPSs (a) from *A. lipoferum* SR42 (*1*), *A brasilense* strains SR50 (*2*), SR80 (*3*), SR88 (*4*), SR109 (*5*), SR111 (*6*), and SR115 (*7*) and (b) Western immunoblotting with Ab against the LPS from *A. brasilense* SR80.

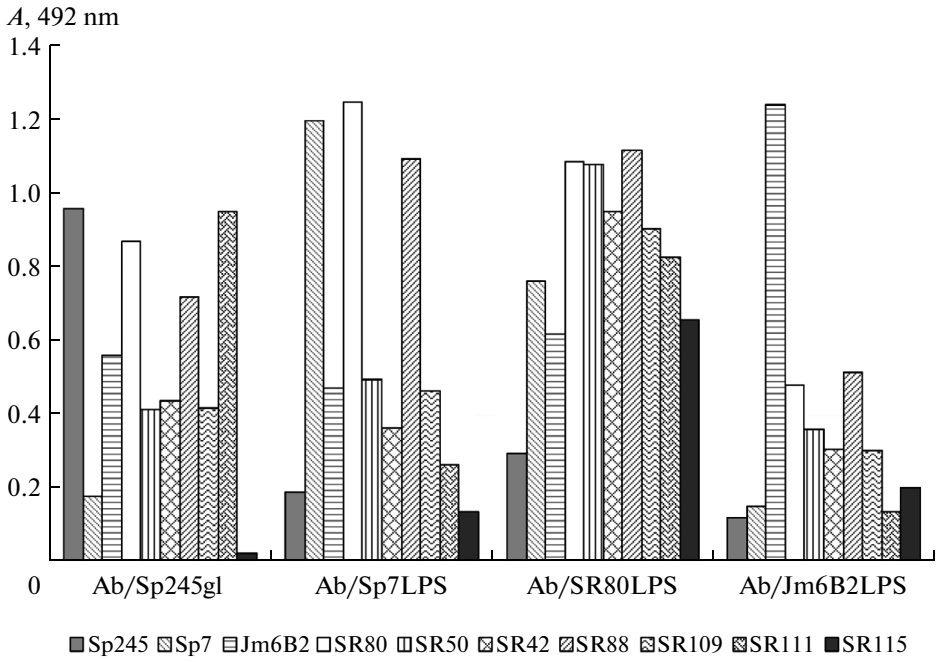

Fig. 2. Comparative immunoenzyme analyses of the LPSs from the studied azospirilla strains with the use of polyclonal antibodies to the LPSs from *A. brasilense* Sp7, SR80, and Jm6B2 and to the glutaraldehyde-treated whole cells of *A. brasilense* Sp245.

Table 3. The data of the ^{13}C- and ^{1}H-NMR spectra of the OPS from *A. lipoferum* SR42 (chemical shifts, ppm)

Monosaccharide residue	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 (5a; 5b) C-5	H-6 (6a; 6b) C-6
OPS1						
→3)-β-D-Gal*p*-(1→ (**A**)	4.67 103.4	3.90 68.5	3.92 77.0	4.26 64.6	3.66 75.9	3.78 62.3
→3)-β-D-Gal*p*NAc*-(1→ (**B**)	4.51 105.4	3.97 52.7	3.95 78.2	4.13 69.8	3.71 76.0	3.78 62.3
→2,3)-α-D-Gal*p*-(1→ (**C**)	5.18 94.3	3.91 74.3	3.90 80.1	4.17 70.8	4.25 71.9	3.72 62.4
α-L-Fuc*p*-(1→ (**D**)	5.25 100.1	3.77 69.0	3.55 71.2	3.71 73.1	4.27 68.2	1.21 16.5
OPS2						
→3,4)-α-L-Fuc*p*-(1→ (**E**)	5.04 98.0	3.91 69.3	3.98 74.8	4.01 81.4	4.38 68.7	1.30 16.2
→4)-β-D-Xyl*p*-(1→ (**F**)	4.45 105.5	3.40 74.8	3.57 75.1	3.68 75.5	3.29; 4.14 63.6	
α-D-Rha*p*-(1→ (**G**)	5.01 102.8	4.02 71.4	3.80 71.4	3.47 73.3	3.92 70.3	1.26 17.9

* Chemical shifts of the *N*-acetyl group signals were 2.03, 23.6, and 175.5 ppm.

A. brasilense Sp7 and Sp245 belong to different serogroups and contain individual antigenic determinants in the OPSs of different structures [24–26]. Immunochemical analysis confirmed the heterogeneity of OPSs in the studied strains.

To elucidate the chemical basis of observed serological cross-reactions from the LPSs of six studied strains, OPSs amounting to 22–27% of the LPS mass were isolated and characterized. Determination of the monosaccharide composition of the OPSs from strains *A. brasilense* SR50, SR88, SR109, SR111, and SR115 and from *A. lipoferum* SR42 by GLC of polyol acetates after complete hydrolysis of the OPS revealed the presence of rhamnose (Rha), fucose (Fuc), xylose (Xyl), galactose (Gal), and *N*-acetylgalactosamine (Gal*N*Ac). Monosaccharide ratios in the OPSs of the studied strains were somewhat different but the ratio of Gal : Gal*N*Ac residues was constant (~1.8 : 1.0) in all samples. These data confirmed our suggestion on the OPS heterogeneity, possibly stemming from the presence of two types of repeating units, which have been earlier identified in *A. brasilense* SR80 [7]. The OPS from strain *A. brasilense* SR115 differed from the others by the presence of glucose (Glc) and increased amount of Rha (by 2- to 3-fold). Determination of the absolute configurations of the monosaccharides by GLC of acetylated octylglycosides revealed the L-configuration of Fuc and the D-configuration of the other monosaccharides.

Chromatography–mass spectrometry of the partially methylated polyol acetates revealed in all the studied OPSs the presence of derivatives of 2,3,4-tri-*O*-methylfucose, 4,6-di-*O*-methylgalactose, 2,4,6-tri-*O*-methylgalactose, and 2-deoxy-4,6-di-*O*-methyl-2-(*N*-methyl)acetamidogalactose (from Gal*N*Ac) as the major components. Besides, 2,3-di-*O*-methylxylose, 2-*O*-methylfucose, and 2,3,4-tri-*O*-methylrhamnose were found in lower amounts. Thus, repeating units of the studied LPSs were represented by branched oligosaccharides having the terminal Fuc and Rha as well as by 2,3-disubstituted Gal and 3,4-disubstituted Fuc at the branching points. The OPS from *A. brasilense* SR115 also contained derivatives of 2-substituted rhamnose, terminal glucose, and minor amounts of 3-substituted rhamnose, 3,4-disubstituted rhamnose, and 2,4-disubstituted rhamnose.

The ^{1}H- and ^{13}C-NMR spectra of the OPSs from strains *A. brasilense* SR88, SR109, and SR111 and from *A. lipoferum* SR42 were identical to each other and contained the signals of different intensity that, together, with the GLC data on the nonstoichiometric ratio of monosaccharides indicated structural heterogeneity of these OPSs.

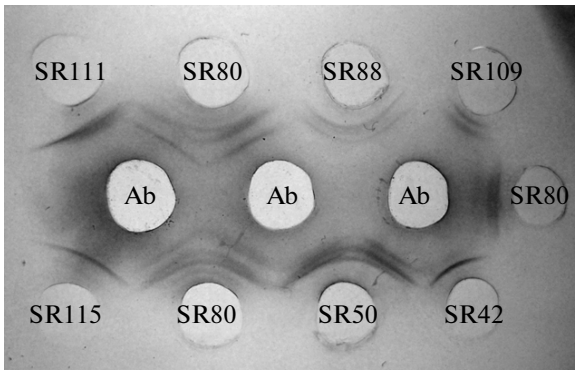

Fig. 3. Dual radial immunodiffusion of the LPSs from the studied *Azospirillum* strains with the antibodies (Ab) to the LPS from *A. brasilense* SR80.

The ^{13}C-NMR spectra of the OPSs from the studied strains (Table 3) contained the signals of seven anomeric carbons at 94.3–105.5 ppm, three CH_3–C groups (C-6 of two Fuc residues and Rha) at δ 16.2–17.9 ppm, three $HOCH_2$–C groups (C-6 of two Gal residues and Gal*N*Ac) at 62.3–62.4 ppm, a nitrogen-bearing carbon (C-2 of Gal*N*Ac) at 52.7 ppm, and the signals of other carbon atoms of the monosaccharide rings at 64.6–81.4 ppm. The spectra also contained the signals of *N*-acetyl group (CH_3 at 23.6 ppm and CO at 175.5 ppm). Signals at 83–88 ppm (at the region typical of furanosides) were not revealed indicating that all the monosaccharide residues were in the pyranose form.

In the region of the anomeric proton resonance of the ^{1}H-NMR spectra (Table 3, Fig. 4a), seven signals at 4.45–5.25 ppm were registered. A high-field region of the ^{1}H-NMR spectra contained the signals of one *N*-acetyl group at 2.03 ppm, two CH_3 groups of the Fuc residues at 1.21–1.30 ppm, and a CH_3 group of the Rha residue at 1.26 ppm, as well as the signals of protons of the monosaccharide rings at 3.29–4.38 ppm (Fig. 4a).

The ^{1}H- and ^{13}C-NMR spectra of the OPSs were assigned using two-dimensional spectroscopy (^{1}H/^{1}H COSY, TOCSY, ROESY, ^{1}H/^{13}C HSQC, and HMBC) (Table 3). It was found that repeating units of the OPSs from strains *A. brasilense* SR88, SR109, and SR111 and from *A. lipoferum* SR42 were identical to those of the OPS from *A. brasilense* SR80, the NMR spectra of which were described in detail earlier [7]. Thus, the carbohydrate part of the LPSs from the studied strains contained either two different polysaccharides, one of which (OPS1) prevailed and another (OPS2) was a minor component or, less probably, two types of repeating units in one polysaccharide molecule of the following structure:

L-Fuc*p*(1α-2)┐
⟶ 3)-α-D-Gal*p*(1 ⟶ 3)-β-D-Gal*p*-(1 ⟶ 3)-β-D-Gal*N*Ac*p*-(1 ⟶ (1)
D-Rha*p*(1α-3)┘
⟶ 4)-α-L-Fuc*p*-(1 ⟶ 4)-β-D-Xyl*p*-(1⟶ (2)

The ^{1}H-NMR (Fig. 4b) and ^{13}C-NMR spectra of the OPS from *A. brasilense* SR115 contained not only the major series of signals described above but also the signals of terminal glucose and several Rha residues, including Rha-2OAc that was in agreement with the data of the monosaccharide composition elucidated by GLC, chromatography–mass spectrometry, and methylation analysis. Assignment of the signals of all monosaccharide residues from the minor polysaccharide of this series was impossible since they overlapped with the signals of the major series. The acetylation of Rha at the second position was confirmed by analysis of integral intensity of the anomeric proton peaks in the ^{1}H-NMR spectrum, the presence of characteristic signals of the *O*-acetyl group in the ^{1}H- and ^{13}C-NMR spectra (CH_3 at 2.20 ppm, CH_3 at 21.6 ppm, and CH_3CO at 174.3 ppm), and considerable shift of the H-2 Rha-2OAc signal at lower field at 5.31 ppm (Fig. 4b). In the ^{1}H, ^{13}C HSQC spectrum, this proton correlated with the carbon at 73.1 ppm. Thus, the OPS from *A. brasilense* SR115 was shown to contain two types of repeating units identical to those of the OPSs from the other studied strains, as well as an additional polysaccharide, the structure of which had not yet been determined.

It should be noted that the fragment [→3)-β-D-Gal*p*-(1 → 3)-β-D-Gal*N*Ac*p*-(1 → 3)-α-D-Gal*p*-(1→] in the main chain of OPS1 from the studied azospirilla strains had been discovered earlier in repeating units of the predominant OPS from *Burkholderia cepacia* of serogroups C and O4, the LPSs of which also contained two types of OPSs [27]. The structure of repeating units of OPS2 was identical to that of the minor OPS from *A. brasilense* Sp7 and was closely related to that of OPS from *A. brasilense* Jm6B2, except for the nonstoichiometric methylation of rhamnose residues in the latter (Table 4).

Thus, the data obtained allow us to conclude that serological similarity of strains *A. lipoferum* SR42 and *A. brasilense* SR80, SR88, SR109, SR111, SR115, and SR50 is due to identical structure of repeating units in

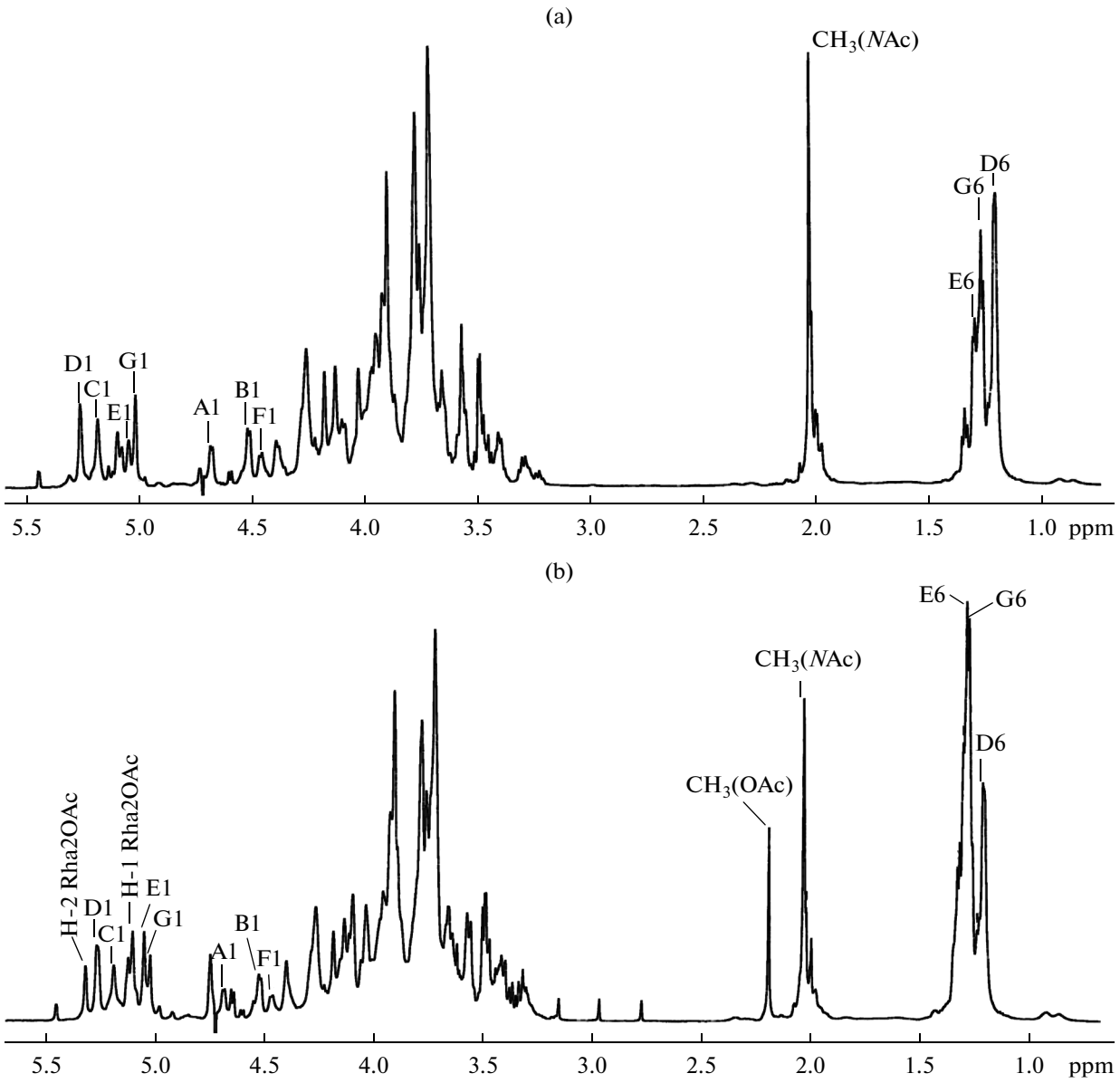

Fig. 4. The ^{1}H-NMR spectra of the OPSs from *A. brasilense* SR109 (a) and SR115 (b).

their OPS. At the same time, the structure of O-antigens in the studied group of strains was not influenced either by species differences or by bacterial associations with different plants realizing C3- or C4-types of photosynthesis. Serological cross-reactions of the strains from this group with the type strain *A. brasilense* Sp7 are based on the presence of identical minor OPSs and common structural fragments in predominant OPSs, such as terminal L-Fuc*p* and β-D-Gal*p* residues (Table 4). The terminal D-Rha*p* residue in OPS2 is possibly responsible for the serological cross-reaction with *A. brasilense* Sp245, a representative of azospirilla serogroup I which is characterized by the presence of homopolymeric D-rhamnan OPS (Table 4). We assume that interstrain variability of the studied *Azospirillum* species, which was determined by immunochemical tests, is due to the individual peculiarities of their LPSs, in particular, to different proportions of the molecules bearing OPS1 or OPS2.

It should be noted that identity of the OPS structures revealed in the studied *Azospirillum* strains had been reported earlier for representatives of serogroups I and III [5, 6]; therefore, molecular mimicry of the bacterial cell surfaces appeared to be widespread. Since bacteria of the genus *Azospirillum* are not characterized by strong specificity for host plants, the revealed wide distribution of certain bacterial surface

Table 4. Structure of the O-specific polysaccharide chains of the LPSs from *A. brasilense* strains

Strain	Structure of the repeating unit
Sp245 [25]	→2)-β-D-Rha*p*-(1 → 3)-α-D-Rha*p*-(1 → 3)-α-D-Rha*p*-(1 → 2)-α-D-Rha*p*-(1 → 2)-α-D-Rha*p*-(1→
Sp7 [26]	L-Fuc*p*(1α–4)┐ →3)-α-L-Rha*p*2OMe*-(1 → 3)-β-D-Gal*p*-(1 → 3)-β-D-Glc*p*NAc-(1→ predominant D-Rha*p*(1α-3)┐ →4)-α-L-Fuc*p*-(1 → 4)-β-D-Xyl*p*-(1→ minor
Jm6B2 [28]	D-Rha*p*3OMe*(1α-3)┐ →4)-α-L-Fuc*p*-(1 → 4)-β-D-Xyl*p*-(1→

* Nonstoichiometric methylation.

glycopolymers may be due to the variability of the mechanisms responsible for the contact interactions of azospirilla with plants and other soil organisms.

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