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EXPERIMENTAL ARTICLES

Chemical and Serological Studies of Liposaccharides of Bacteria of the Genus *Azospirillum*

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Abstract—The analysis of the lipopolysaccharides (LPS) of nine strains of azospirilla revealed the presence of the characteristic components of these glycopolymers: carbohydrates, hydroxylated fatty acids, and 2-keto-3-deoxyoctonic acid (KDO). SDS electrophoresis revealed the heterogeneous nature and the strains differences in the ratio of the molecular S and R forms present in the LPS. Polyclonal rabbit antibodies (Ab) were obtained against the isolated LPS_{Cd}, LPS_{Sp59b}, LPS_{Sp7}, LPS_{S17}, and LPS_{KBC1} preparations. Based on the results of the serological studies of the LPS, the bacterial strains investigated in the work were divided into two main serogroups. Based on the immunoblotting data, Ab_{Sp59b} and Ab_{Cd} were found to be formed in response to both the S and R forms of the LPS molecules, whereas all the rest formed in response to the S forms only. It was shown that the heterogeneity of the antigenic determinants is typical of the second LPS group. It was suggested that rhamnose plays one of the key roles in the specific interactions between the azospirillum membrane LPS and Ab.

Key words: azospirillum, lipopolysaccharide, serological studies.

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Among the bacteria carrying out associative interactions with plant roots, *Azospirillum* spp. assigned to the α subclass of the *Proteobacteria* are being actively studied. These microorganisms belong to the group of rhizobacteria stimulating plant growth owing to their high nitrogen-fixing activity and their capacity for producing phytohormones and other active substances, such as polysaccharides (PS) and polysaccharide-containing polymers [1, 2]. New species are often added to the genus *Azospirillum*: over the last three years, three new species have been added to the previously known seven [3–5]. In this regard, the urgency of the studies aimed at developing quick and effective methods for differentiation between microorganisms based on serodiagnostics is ever increasing. Earlier, it was attempted to identify the strains of azospirilla isolated from the surface of plant roots and from root tissues using polyspecific antibodies [6]. However, the data interpretation was complicated by cross reactions, more likely, between the protein antigens. The application of the hybridoma technique led to the acquisition of the monoclonal bodies against several *A. brasilense* strains; this approach revealed noticeable interstrain differences in the way of colonizing the wheat roots by bacteria [7].

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In order to differentiate between azospirilla with certainty, additional information is urgently required on the chemotaxonomy and serology of these microorganisms. Usually, in prokaryote systematics, a number of categories are considered [8], including the serological (agglutination, immunodiffusion) and chemotaxonomic ones (including the LPS electrophoretic profile).

A number of works employing the antibodies (Ab) against whole bacterial cells treated with glutaraldehyde have been devoted to the immunochemical properties of the cell surface of azospirilla [9, 10]. However, a detailed serological classification of azospirilla has not yet been developed.

This work was carried out in the course of the systematic study of bacteria of the genus *Azospirillum*; its aim was to determine the serological and chemotaxomonic characteristics of the LPS of nine azospirillum strains assigned to the species *A. brasilense, A. irakense*, and *A. lipoferum.*

MATERIALS AND METHODS

Cultivation conditions. The bacterial strains of the genus *Azospirillum* used in the work are listed in Table 1. All the microorganisms were cultivated in a

Table 1. Microorganisms used in the work

Strain	Host plant	Strain donor	Reference
		A. brasilense	
Sp7 (ATCC 29145)	Crabgrass (*Digitaria decumbens*)	D. Janssens, Rijksuniversiteit Gent. Lab.Voor. Microbiol. Gent, Belgium	[11]
Cd (ATCC 29710)	Bermuda grass (*Cynodon dactilon*)	Y. Bashan, The Center for Biological Research of the Northwest, Mexico	[12]
Sp245	Wheat (*Triticum aestivum*)	J. Döbereiner, Empresa Brasileira Pesquisa Agropecua ria, Rio de Janeiro, Brazil	[13]
S17	Bajree (*Perl millet*)	N.A. Lahiri, Division of Soilater-Plant Relationship, Central Arid Zon Institut, Jodhpur, India	–
SR75	Wheat (*Triticum aestivum*)	Isolated from Saratov oblast cereals by research workers from the IBPPM, Russ. Acad. Sci. (Saratov)	[14]
SR15	Cock's-foot grass (*Dactylis glomerata*)		
		A. lipoferum	
Sp59b (B-1519) (ATCC 29707)	Wheat (*Triticum aestivum*)	VKM	[11]
RG20a (ATCC 29708)	Wheat (*Triticum aestivum*)	J. Döbereiner, Empresa Brasileira Pesquisa Agropecua ria, Rio de Janeiro, Brazil	[15]
		A. irakense	
KBC1 (CIP103311)	Rice (*Oryza sativa*)	C. Elmerich, Inst. Pasteur, Paris, France	[16]

liquid malate–salt medium with vitamins [2] at 30°C until the end of the exponential growth phase.

After precipitating the cells by centrifugation, a capsule was removed from their surface, as described in [17]; after that the cells were treated with acetone three times and air-dried.

LPS isolation from the external membrane of the acetone-dried bacterial mass (20 g) was carried out by extraction with hot 45% aqueous phenol using the Westphal method [18]. After phenol removal by dialysis, the aqueous fractions of the extracts were concentrated and purified by gel filtration on a column with Sepharose CL-4B (55 × 1.8 cm, V_0 = 40 ml); 0.025 M NH_4HCO_3 (pH 8.3) was used as an eluent. The detection of the separation products in the eluates was carried out using an LKB 2142 differential flow refractometer (LKB, Sweden). All the carbon-containing fractions, which did not exhibit absorption in the 240–260-nm range, were pooled, concentrated, and lyophilized.

The colorimetric determination of carbon, 2-keto-3-deoxyoctonic acid (KDO), proteins, nucleic acids, and phosphorus in the LPS preparations was carried out according to the conventional methods described earlier [2]. The measurements were performed using an SF-46 spectrophotometer (LOMO, Russia).

Electrophoresis of the LPS preparations was carried out in 12.5% polyacrylamide gel with sodium dodecyl sulfate (SDS-PAAG) [19]. The LPS were visualized by staining the gels with silver nitrate [20].

For **immunoblotting**, the separated components were electrically transferred onto the nitrocellulose filters (Sigma, 0.2 μm). Immunodetection was carried out by incubating the blots with the anti-LPS Ab; horse radish peroxidase conjugated with antirabbit goat antibodies and 3,3-diaminobenzidine were used for development.

For **obtaining the antibodies,** the rabbits were immunized thrice at two-week intervals by sequential injections of 0.5, 1.0, and 1.5 mg of LPS into their popliteal lymph nodes. During the first immunization, the antigen was mixed in the ratio 1 : 1 with a complete Freund adjuvant; subsequent immunizations were performed with an incomplete adjuvant. The blood was collected in a week after the last immunization.

The immunoglobulin G fractions were obtained from the antisera by precipitation with ammonium sulfate [21].

The agglutination reaction was performed in a 96-well plate for immunological reactions. Before staging the experiment, the complement was inactivated in the antiserum by heating it at 56°C for 30 min. The bacterial cells were suspended in the phosphate buffer (pH 7.2) with 0.12 M NaCl; their concentration was adjusted to the values of A_{660} = 1 (l = 1 cm). The antiserum titer was determined as its maximum dilution at which the bacterial cell agglutination was visualized in the form of an irregular precipitate at the bottom of the plate well.

The immunodiffusion assay was performed according to the standard technique in 1% agarose gels.

Table 2. LPS biopolymer composition of bacteria of the genus *Azospirillum*

Strains		LPS yield, % of the dry cell weight	Content, %			
			Carbohydrates	Proteins	KDO	Phosphorus
A. brasilense	SR75*	1.0	53.2 ± 0.2	2.1 ± 0.1	3.3 ± 0.1	0.1
	S17	1.7	29.0 ± 1.3	0.8 ± 0.4	0.3	3.3 ± 0.2
	Cd**	3.2	28.4 ± 2.1	0.3 ± 0.1	3.5 ± 0.1	1.9 ± 0.1
	Sp7	2.8	21.9 ± 0.3	0.6 ± 0.1	0.6	2.5 ± 0.2
	SR15	1.1	24.5 ± 3.6	5.4 ± 0.2	0.2	2.6 ± 0.2
A. lipoferum	Sp59b	2.6	38.8 ± 1.4	2.4 ± 0.2	4.4 ± 0.1	0.5
	RG20a	1.4	48.3 ± 3.3	2.3 ± 0.1	2.8 ± 0.1	–
A. irakense	KBC1	1.1	62.3 ± 2.7	0.9 ± 0.1	0.7	3.4 ± 0.8

Notes: "–" The component was not found.
* The data from [22].
** The data from [23].

The tested samples (14 μl each) were introduced into the 4-mm wells. The LPS were introduced into the wells at a concentration of 1 mg/ml, and the antibodies at 15 mg protein per 1 ml. The precipitate formed was stained with Coomassie R-250.

The enzyme linked immunosorbent assay (ELISA) was performed in 96-well polystyrene plates for ELISA. Hydrogen peroxide with *o*-phenylenediamine was used as a substrate reagent. The optical density measurements of the tested samples were carried out at 490 nm using an AIF-Ts-01S immunoenzyme analyzer (ZAO ILIP, St. Petersburg).

The results of all the experiments were processed statistically. The data are presented as the means (of at least three experiments each of which was made with three replicas) with rms error.

RESULTS AND DISCUSSION

The LPS of *A. brasilense* Sp245, S17, Sp7, Cd, SR75, SR15, *A. irakene* KBC1, and *A. lipoferum* Sp59b, RG20a were isolated by extraction according to Westphal. The LPS yield varied between 1 and 3% of the dry microbial mass weight, depending on the strain (Table 2). The chemical analysis showed that the use of chromatographic methods for extract fractionation allowed us to achieve a high degree of LPS purification, which was confirmed by the trace amounts of nucleic acids in the samples (less than 0.1%). Most of the preparations obtained contained an insignificant amount of the protein impurities that were not visualized by staining with Coomassie R-250 when the LPS were analyzed by electrophoresis in SDS-PAAG. An exception was the LPS_{SR15} preparation, in which 5.4% of the protein was identified colorimetrically, and the band corresponding in color to the protein components was visualized in the electrophoregram. However, NMR spectroscopy confirmed the presence of a substitute of peptide nature in the composition of the O-antigen polysaccharide portion as a specific feature of this preparation (unpublished data); this was possibly the reason for the overestimated protein content.

When the biopolymer composition was studied, it was shown that carbohydrates accounted for 22 to 62% of the mass of the LPS preparations. Saturated, unsaturated, and hydroxy acids with a C_{10}–C_{19} chain length were identified in the composition of the LPS lipid portion. The major (in terms of content) fatty acids were 3-hydroxytetradecanoic, hexadecanoic, 3-hydroxyhexadecanoic, and octadecenic.

KDO, the only structural element invariably present in LPS, was identified in all the LPS. However, while its content in LPS_{SR75}, LPS_{Cd}, LPS_{Sp59b}, and LPS_{RG20a} was approximately 3–4%, for the LPS of the other strains it was less than 1% (Table 2). In addition, significant interstrain differences were revealed in the total phosphorus content in the above LPS; this parameter is known to influence significantly the submolecular LPS organization in the membrane. The scatter in the phosphorus content in the samples may result from the differences in the degree of phosphorylation of the core oligosaccharides and lipid A glucosaminebiose.

The phosphate groups attached to KDO are known to screen the acid-labile bond, hindering LPS hydrolysis and, accordingly, KDO determination. This may be the reason for the low KDO content reported earlier for the LPS of certain *A. brasilense* and *A. lipoferum* strains [24] and shown in our work for LPS_{Sp7}, LPS_{KBC1}, LPS_{S17}, and LPS_{SR15}.

The electrophoretic LPS profile, along with the chemical composition, is a generally accepted chemotaxonomic criterion, which is often used to establish a relationship between large bacterial taxa and between the species and strains inside individual genera. It is known that LPS may be represented by the S and R molecular forms. The S form molecules include lipid A, the core oligosaccharide, and the O-specific polysaccharide (OPS), whereas the R form is devoid of OPS and includes only lipid A and the core oligosaccharide. Although the wide OPS variation in the number of

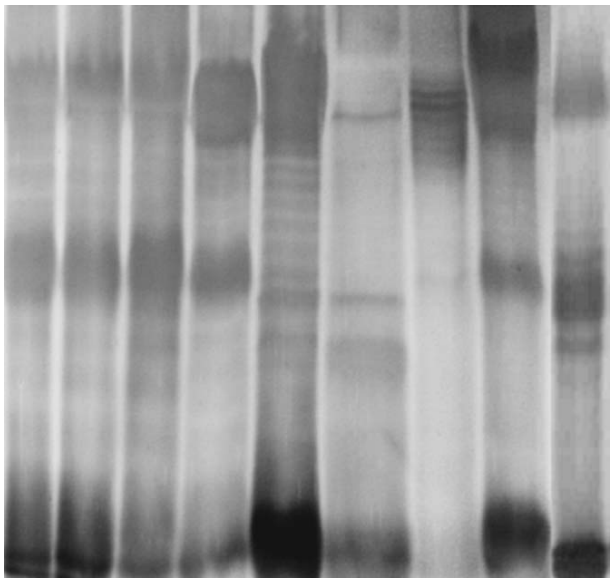

Fig. 1. PAAG electrophoresis of LPS preparations from (*1*) *A. brasilense* Sp245, (*2*) SR75, (*3*) *A. lipoferum* RG20a, (*4*) *A. brasilense* Sp7, (*5*) Cd, (*6*) *A. lipoferum* Sp59b, (*7*) *A. irakense* KBC1, (*8*) *A. brasilense* S17, and (*9*) SR15.

repeating links and the component composition leads to substantial differences in the LPS migration in SDS-PAAG electrophoresis, the electrophoretic LPS profile is characteristic of individual species and even strains [8].

The data obtained as a result of electrophoretic separation clearly demonstrate the heterogeneous nature of the *Azospirillum* LPS (Fig. 1). All the LPS studied are represented by a wide range of molecules of different sizes. The strains *A. brasilense* Sp245, SR75, Sp7, S17, and SR15 and *A. lipoferum* RG20a (Fig. 1, lanes *1–4, 8*, and *9*) have a similar electrophoretic LPS profile characterized by the equivalent presence of the S and R form molecules. The latter, as shown earlier for the type strain *A. brasilense* Sp7 [9], are concentrated in the middle part of the electrophoregram. The LPS S forms (S-LPS) of Sp245, SR75, and RG20a were found to contain two main groups of molecules differing in mobility in the electric field, whereas the LPS R forms (R-LPS) of these strains, as well as the S- and R-LPS of strains Sp7 and S17 and the S-LPS of strain SR15, were characterized by the same mobility in the gel. However, the R-LPS_{SR15} electrophoregram showed the presence of the second minor, clearly defined band (Fig. 1, lane *9*).

The LPS preparations of the other strains differed significantly in the electrophoretic profile from the aforementioned ones. The strain KBC1 (Fig. 1, lane *7*) was characterized by the predominance of the S-LPS_{KBC1} molecules. As seen from the upper part of the electrophoregram, they formed a series of bands, probably corresponding to molecules differing in length per one repeating link in the OPS composition.

In the LPS_{Cd} preparation, the S form also dominated over the R form (Fig. 1, lane *5*). The R-LPS_{Cd} molecules were close in mobility in the electric field to the dominant R-LPS_{Sp59b} molecules represented in the electrophoregram by two intense bands (Fig. 1, lanes *5, 6*).

To reveal the serological relationship between the azospirilla strains studied, we obtained the Ab against the bacterial LPS preparations of *A. irakense* KBC1, *A. brasilense* Sp7, S17, and *A. lipoferum* Sp59b; three of these strains are the type ones for the corresponding species. It should be noted that for *A. irakense* KBC1, *A. lipoferum* Sp59b, and *A. brasilense* S17, the structures of the repeating OPS links have been determined by the time the work was started [25, 26; for *A. brasilense* S17, the data are not published]. The IgG fractions (Ab_{KBC1}, Ab_{Sp7}, Ab_{S17}, and Ab_{Sp59b}, respectively) known to be characterized by specificity to the LPS polysaccharide portion were isolated from the sera by precipitation with ammonium sulfate [27]. Using them, it is possible to reveal the homologous antigenic determinants in the LPS of both azospirilla and, probably, related rhizobacteria.

All the LPS studied in the course of this work were tested with the immunodiffusion and the enzyme-linked immunosorbent assay for the presence of serological cross reactions with the anti-LPS Ab obtained earlier and the Ab against whole *A. brasilense* Sp245 (Ab_{Sp245}) cells treated with glutaraldehyde [10] (Fig. 2, Table 3).

The immunodiffusion method with the homologous antibodies revealed at least one thermostable antigenic determinant in the LPS_{Sp59b} composition (Fig. 2a), at least two in the LPS_{Sp7} and LPS_{S17} (Fig. 2b, c), and at least three in the LPS_{KBC1} (Fig. 2d). The results of ELISA demonstrating the Ab specificity to different LPS are shown in Table 3.

The comparative immunodiffusion assay with Ab_{Sp245} revealed a serological relation between the LPS of *A. brasilense* Sp245, SR75, and SR15 and *A. lipoferum* RG20a (Fig. 2f). The interaction between the LPS_{SR75} and these Ab resulted in the formation of two precipitation bands, which completely merged with those characteristic of LPS_{Sp245}. The LPS_{RG20a} gave a cross reaction with only one of the LPS_{Sp245} antigenic determinants corresponding to the external precipitation band. The results of ELISA showed that Ab_{Sp245} revealed the highest specificity to the homologous LPS and to LPS_{SR75} and a less markedly pronounced homology to LPS_{RG20a} (Table 3). The immunochemical differences of LPS_{RG20a} from LPS_{Sp245} and LPS_{SR75} (considering that they had an identical electrophoretic profile as evidenced by the electrophoretic data) may be explained by the weak immunogenicity of one of the LPS_{RG20a} determinants.

ELISA and immunodiffusion confirmed the absence of interaction between Ab_{Sp245} and LPS_{SR15}. With a fourfold increase in the antigen concentration in the immunodiffusion reaction, crossing with the internal

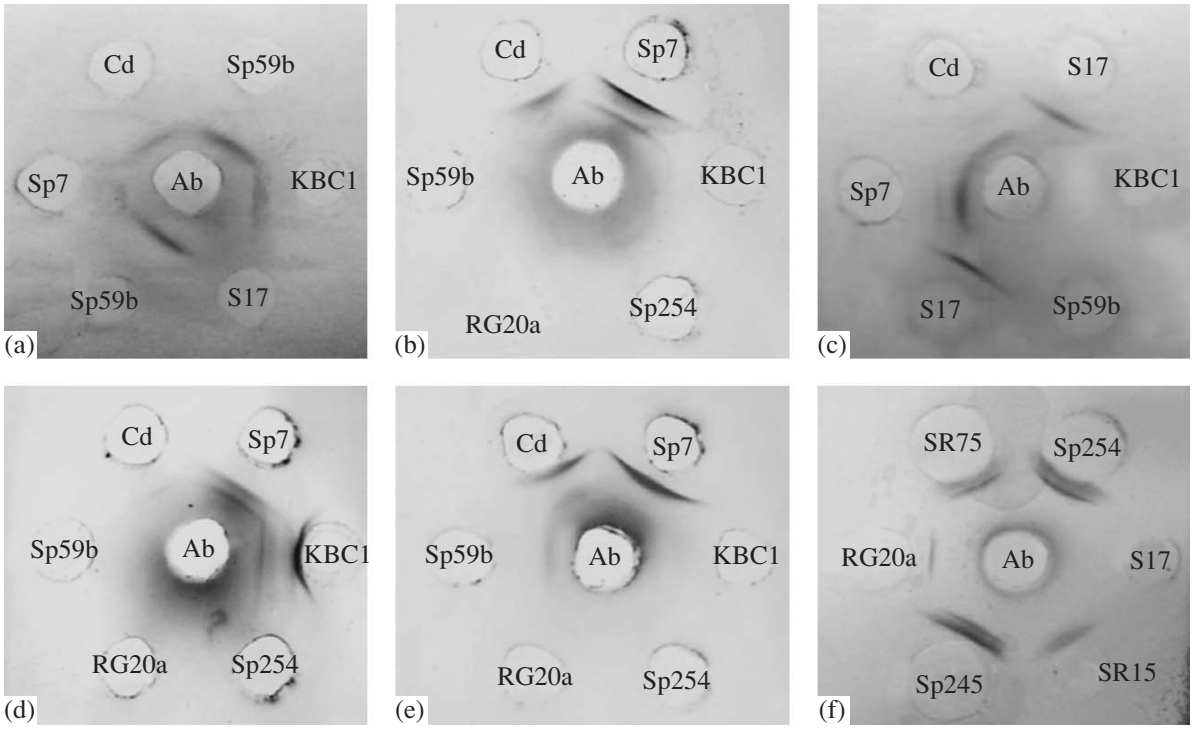

Fig. 2. Double immunodiffusion of the LPS preparations from *A. brasilense* S17, Sp7; Cd, *A. lipoferum* Sp59b, and *A. irakense* KBC1 with Ab_{Sp59b} (a); *A. brasilense* Sp245, Sp7, Cd, *A. lipoferum* Sp59b, RG20a, and *A. irakense* KBC1 LPS preparations with Ab_{LPSSp7} (b); Ab_{S17} (c); $Ab_{LPSKBC1}$ (d); Ab_{LPSCd} (e); and *A. brasilense* Sp245, SR75, S17, SR15 and *A. lipoferum* RG20a LPS with Ab_{245} (f).

precipitation band was noted. Based on this, it may be suggested that the corresponding antigenic determinants are insignificantly represented in LPS_{SR15} (Fig. 2f).

Apart from LPS_{Sp7}, Ab_{Sp7} interacted only with LPS_{Cd} both in immunodiffusion and ELISA. Only the external precipitation bands merged in immunodiffusion (Fig. 2b), which suggests a cross reaction with only one of the LPS_{Sp7} antigenic determinants. The results of ELISA demonstrated the highest specificity of Ab_{Sp7} precisely to LPS_{Sp7} (Table 3).

Both Ab_{Sp59b} and Ab_{KBC1}, apart from the homologous LPS, revealed specificity to LPS_{Cd} and LPS_{Sp7}. It was shown that Ab_{Sp59b} did not interact with LPS_{KBC1},

Table 3. Results of the direct and cross enzyme-linked immunosorbent assay of the LPS of bacteria of the genus *Azospirillum* with the antibodies obtained against LPS_{S17}, LPS_{Cd}, LPS_{Sp7}, LPS_{KBC1}, and LPS_{Sp59b}, and against whole Sp245 cells treated with glutaraldehyde

LPS \ Antibodies	$Cells_{Sp245glut}$	LPS_{S17}	LPS_{Cd}	LPS_{Sp7}	LPS_{KBC1}	LPS_{Sp59b}
A. brasilense Sp245	+++	+	–	–	–	–
A. brasilense SR75	+++	+	–	–	–	–
A. lipoferum RG20a	++	–	–	–	–	–
A. brasilense S17	–	+++	+	–	–	–
A. brasilense Cd	–	++	+++	++	+	+++
A. brasilense Sp7	–	+++	+++	+++	+++	+
A. irakense KBC1	–	–	–	–	+++	–
A. lipoferum Sp59b	–	–	++	–	–	+++
A. brasilense SR15	–	–	–	–	–	–

Notes: D_{490} not higher than 0.3, no interaction (–); D_{490} 0.3–0.6, very weak interaction (+); D_{490} 0.6–1.0, moderate interaction (++); D_{490} above 1.0, strong interaction (+++).

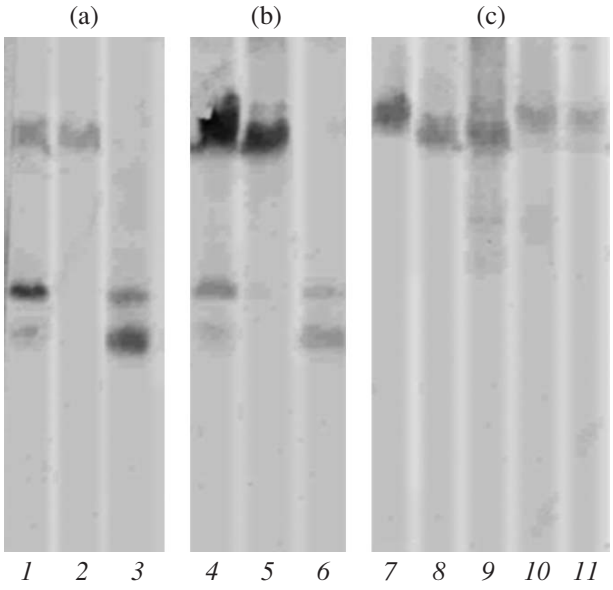

Fig. 3. Western immunoblotting of the LPS_{Cd} (*1, 4, 8*), LPS_{Sp7} (*2, 5, 9*), LPS_{Sp59b} (*3, 6*), LPS_{S17} (*7*), LPS_{Sp245} (*10*), and LPS_{SR75} (*11*) preparations with the use of rabbit Ab_{Sp59b} (a), Ab_{Cd} (b), and Ab_{S17} (c).

and Ab_{KBC1} did not interact with LPS_{Sp59b}, whereupon we can speak about the ability of these Ab to detect different antigenic determinants in LPS_{Cd} and LPS_{Sp7}.

The results of immunodiffusion of Ab_{Sp59b} with LPS_{Sp59b}, LPS_{Cd}, and LPS_{Sp7} demonstrate complete merging of the precipitation bands (Fig. 2a), which indicates the presence of shared determinants in the composition of these antigens. The ELISA of these preparations showed that LPS_{Sp59b} and LPS_{Cd} were closer to each other antigenically than to LPS_{Sp7} (Table 3). As was noted earlier (see above), the LPS_{Sp59b} and LPS_{Cd} R forms also had the same mobility in an electric field (Fig. 1).

The immunodiffusion assay with Ab_{KBC1} revealed LPS_{Sp7} crossing with two antigenic determinants revealed in LPS_{KBC1}, and LPS_{Cd} crossing with only one of them (Fig. 2d). It should be mentioned that LPS_{KBC1} had its own antigen, which corresponded to an individual band in immunodiffusion (Fig. 2d). In ELISA, Ab_{KBC1} revealed a higher specificity to LPS_{Sp7} than to the homologous LPS (Table 3). Such a phenomenon was recorded earlier for Ab_{Cd} [23], which interacted with LPS_{Sp7} more actively than with LPS_{Cd}. This result may be explained by the presence of a greater number of specific antigenic determinants in LPS_{Sp7} compared to the corresponding LPS. It was shown in the course of this work that Ab_{Cd} interacted with LPS_{S17} rather weakly (Table 3).

In ELISA, Ab_{S17}, in addition to the homologous LPS, interacted with LPS_{Cd} and LPS_{Sp7}, showing high specificity to the latter (Table 3). It is necessary to point out that LPS_{Sp7} and LPS_{S17} had similar electrophoretic profiles (Fig. 1). Moreover, the results of immunodiffusion (Fig. 2c) with Ab_{S17} showed that the LPS_{S17} precipitation band did not completely merge with the external LPS_{Sp7} and LPS_{Cd} precipitation bands, forming spurs, which indicates the presence of individual antigenic determinants in the LPS_{S17} composition.

The results of ELISA showed very slight interaction of Ab_{S17} with LPS_{Sp245} and LPS_{SR75} (Table 3) not detected by immunoduffusion, which is likely to be linked to the scanty representation of the specific determinants.

The fact that the antibodies formed against LPS are characterized by specificity to its polysaccharide part allows us to state that the serological cross reactions revealed in this work indicate the presence of shared structural fragments in the corresponding OPS.

Based on the data obtained, all the strains were divided into two serogroups by LPS immunospecificity. The first serogroup included the strains *A. brasilense* Sp245, SR75, and SR15 and *A. lipoferum* RG20a; the second serogroup, *A. brasilense* Sp7, *A. irakense* KBC1, *A. brasilense* Cd, and *A. lipoferum* Sp59b. The strain *A. brasilense* S17 was tentatively assigned to the second serogroup. Taking into account the results of the immunochemical studies, as well as the structural peculiarities of OPS_{S17} (unpublished material), this strain may later be identified as a separate serogroup.

Analysis of the results of the serological studies enabled us to reveal different determinants in the composition of the LPS preparations of the second serogroup strains. We believe that some of them are characteristic of *A. lipoferum* Sp59b, *A. brasilense* Sp7, and *A. brasilense* Cd; the other, of *A. irakense* KBC1, *A. brasilense* Sp7, and *A. brasilense* Cd (different from the determinants shared with *A. lipoferum* Sp59b).

The next stage in our work was to pinpoint the localization of the determinants to which the Ab are specific and with which immunological cross reactions could occur. The antigenic determinants concentrated in the LPS S and R forms were designated as the S- and R-antigens, respectively. The results of immunoblotting showed that Ab_{Sp59b} and Ab_{Cd} are specific to the S and R molecular forms (Figs. 3a, 3b). As is seen from Figs. 3a and 3b, LPS_{Sp59b} crosses serologically with the LPS_{Cd} S- and R-antigens and LPS_{Sp7} only with LPS_{Cd} S-antigens.

Earlier, we showed the identity of the repeating OPS links from LPS_{Cd} and LPS_{Sp59b} [23, 26], which confirms the serological crossing with the S-antigen. The differences in the immunodiffusion patterns of the corresponding LPS with Ab_{Cd} may be explained by the S form molecules being less abundant in the LPS_{Sp59b} preparation.

The results of immunoblotting of LPS_{S17}, LPS_{Sp7}, LPS_{Cd}, LPS_{Sp245}, and LPS_{SR75} with Ab_{S17} showed that these Ab were specific to the S form molecules (Fig. 3c); consequently, these strains cross serologically with the S-antigens as well. LPS_{Sp245} and LPS_{SR75}

are known to have an identical structure of the repeating OPS link, namely, linear penta-D-rhamnane [22]. The structure of OPS_{Cd} is radically different from the above-mentioned one and appears to be a digalactan with a mannorhamnan side chain [23], with rhamnose in the L form. According to unpublished data, LPS_{S17} and LPS_{Sp7} also have rhamnose as an OPS constituent part. Based on these data, it may be suggested that rhamnose is one of the key sugars recognized by the Ab obtained against purified LPS preparations. The presence of rhamnose in a D or L form, as well as different substitution positions, may substantially influence the degree of its interaction with Ab.

Thus, the LPS analysis demonstrated the presence of such characteristic components as carbohydrates, hydroxylated fatty acids, and KDO. Polyclonal rabbit Ab obtained against the isolated LPS_{Sp59b}, LPS_{Sp7}, LPS_{S17}, and LPS_{KBC1} preparations may be used, apart from serological studies, for immunological identification of these microorganisms. Basing on the results of the serological studies, the strains described in this work were divided into two serogroups. It was suggested that rhamnose may play one of the key roles in specific interactions between the azospirillum membrane LPS and Ab.

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