ISSN 0026-2617, Microbiology, 2014, Vol. 83, No. 1–2, pp. 102–109. © Pleiades Publishing, Ltd., 2014.

EXPERIMENTAL ARTICLES

Serological Relationships of Azospirilla Revealed by Their Motility Patterns in the Presence of Antibodies to Lipopolysaccharides

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Received July 30, 2013

Abstract—Motility of the serologically different *Azospirillum brasilense* strains Sp245 (serogroup I) and Sp7 (serogroup II) was studied in the presence of antibodies to their lipopolysaccharides (LPS). A procedure was proposed in order to determine the motility patterns indicating the specificity of the interaction between the anti-LPS antibodies and bacteria. Analysis of the effect of such antibodies on motility of 25 strains (*A. brasilense*, *A. lipoferum*, *A. irakense*, and *Azospirillum* sp.) revealed bacteria exhibiting antigenic cross reactions with *A. brasilense* Sp7 or Sp245. The effect of anti-LPS antibodies on motility of azospirilla was in agreement with the results of immune agglutination analysis of bacterial cells and of immunodiffusion analysis of the LPS preparations. According to our results, strains *Azospirillum* sp. SR81 and *A. brasilense* SR14 should be included into serogroups I and II, respectively.

Keywords: *Azospirillum*, motility, O antigen, antibodies, serogroup, serological analysis

DOI: 10.1134/S0026261714020179

The present work deals with gram-negative associative bacteria of the genus *Azospirillum*, which act as stimulators of plant growth [1]. Three serogroups of *Azospirillum* have been identified for the time being as a result of studying the serological properties and structures (chemotypes) of lipopolysaccharides (LPS, O antigens) [2–4]. These are the major components of the outer membrane of gram-negative bacteria, usually containing the antigen determinants responsible for the serological specificity of bacteria and available for the interaction with the cells of other organisms. The O-specific polysaccharides (OPS) of the LPS of serogroup I azospirilla are linear D-rhamnans cross-reacting with the antibodies (Ab) specific to LPS of the strain *A. brasilense* Sp245 [2, 3]. Serogroup II *Azospirillum* strains are characterized by the presence of heteropolysaccharide OPS cross-reacting with the Ab against LPS of the type strain *A. brasilense* Sp7 [2, 5]. Serogroup III comprises *Azospirillum* strains that show serological affinity to the type strain *A. lipoferum* Sp59b. The chemical basis of the serological relationship between azospirilla comprising this serogroup is the common oligosaccharide motif formed by three L-rhamnose residues [4]. The presence of similar antigens within somatic LPS, capsular polysaccharides, and exopolysaccharides has been demonstrated for the strains *A. brasilense* Sp7 and Sp245 [6]. The cells of *Azospirillum* maintained in liquid media have a single polar flagellum responsible for motility and chemotaxis, the important factors for efficient colonization of plants by these bacteria [1, 7]. The polar flagellum of *A. brasilense* strains Sp7 and Sp245 is covered with a sheath formed of LPS molecules, which is typical of some other bacteria [8–10].

The goal of the present work was to develop a test procedure for express detection of antigen chiasms between the strains for taxonomic studies, which is based on the changing motility characteristics of microorganisms in the presence of antibodies to the surface structures of bacterial cells.

MATERIALS AND METHODS

The strains are listed in Table 1.

Azospirilla were cultivated in a malate-salt medium (MSM) [17]. The bacteria were grown for 18 h in liquid MSM at 30°C.

LPS were extracted with ethylenediaminetetraacetic acid (EDTA) disodium salt [10]. Bacterial cells were washed in a phosphate–saline buffer (PSB; pH 6.8) and precipitated by centrifugation; LPS were extracted for 30 min at room temperature in the buffer (pH 8.5) containing 0.1 M Tris-HCl, 10 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), and 1% Triton X-100 (EDTA concentration was 0.05 mM per 1 g of wet cells). The cells were removed from the extract by centrifugation.

Anti-LPS antibodies were obtained as described [18]. The Ab were conjugated with gold nanoparticles for immunoelectron microscopy by the method described [19].

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Table 1. *Azospirillum* strains used in the work

Strain	Characteristic	Source
	Azospirillum brasilense	
Cd	Wild type, isolated in the USA from Bermuda grass roots after inoculation with Sp7	[11]
S27	Wild type, isolated in India from the roots of the shrub *Sericostoma pauciflorum*	A.L. Lahiri, CAZRI, Jodhpur, India
Sp7	Wild type, isolated in Brazil from Pangola grass rhizosphere	[12]
Sp107	Wild type, isolated in Brazil from wheat roots	[13]
Sp245	Wild type, isolated in Brazil from wheat roots	[14]
SR8	Wild type, isolated in Russia from Hungarian brome grass roots	[15]
SR14	Wild type, isolated in Russia from Hungarian brome grass roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR15	Wild type, isolated in Russia from cocksfoot roots	[15]
SR32	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR41	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR50	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR55	Wild type, isolated in Russia from wheat roots	[15]
SR64	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR72	Wild type, isolated in Russia from wheat roots	[15]
SR75	Wild type, isolated in Russia from wheat seedlings	[15]
SR80	Wild type, isolated in Russia from wheat seedlings	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR87	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
SR88	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
	Azospirillum sp.	
SR81	Wild type, isolated in Russia from wheat roots	L.I. Pozdnyakova, IBPPM RAS, Saratov, Russia
	Azospirillum irakense	
KA3	Wild type, isolated in Iraq from rice rhizosphere	[16]
KBC1	Wild type, isolated in Iraq from rice roots	[16]
	Azospirillum lipoferum	
Sp59b	Wild type, isolated in Brazil from wheat roots	[12]
SpRG20a	Wild type, isolated in Brazil from wheat roots	[12]

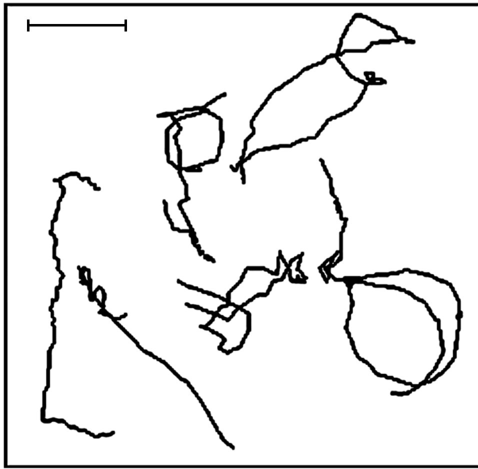

Fig. 1. The trajectories of movement of *A. brasilense* Sp245 cells grown in liquid MSM. Scale bar is 10 μm.

Double immunodiffusion was performed according to the standard method [2] in 1% agarose gels. The gels were stained with Coomassie R-250.

For agglutination reaction, 18-h culture cells were washed by centrifugation and resuspended in PSB to the values $A_{600} = 1.0–1.2$ ($l = 1$ cm). Cell suspensions (50 μL) were dispensed into 96-well plates for immunological reactions with the equal volumes of serial dilutions of anti-LPS Ab solutions (the initial Ab concentration was 100 μg/mL) in PSB. The reaction was considered positive if irregular-shaped precipitate was formed on the bottom of the respective well.

Bacterial motility in liquid media was studied in wet mount or hanging drop preparations obtained from cell suspensions (A_{600} = 0.5) prepared as described above and mixed with Ab (1 : 1) (the final Ab concentrations are given in Results and Discussion). Microscope slides were examined in a JENAVAL transmission microscope (Carl Zeiss Jena, Germany) under phase contrast using an objective with a wide field of view. Observations were recorded with a DCR-TRV900E video camera (SONY, Japan). The motility of all cells in the field of view of the microscope was estimated, with determination of the average rate of movement in 50–150 randomly selected motile cells. The video image was analyzed using a computer program developed by V.A. Krestinenko (Institute of Biochemistry and Physiology of Plants and Microorganisms, Russian Academy of Sciences), which marks the position of a cell in Cartesian coordinates in the frame mode [20].

Electron microscopic analysis of bacterial cells was performed with Libra 120 (Carl Zeiss, Germany) at an accelerating voltage of 120 kV.

The results were statistically processed using Microsoft Office Excel 2007. Confidence intervals were determined for the 95% significance level.

RESULTS AND DISCUSSION

Effect of anti-LPS antibodies on bacterial motility. *Azospirillum* cells grown in liquid medium have a long

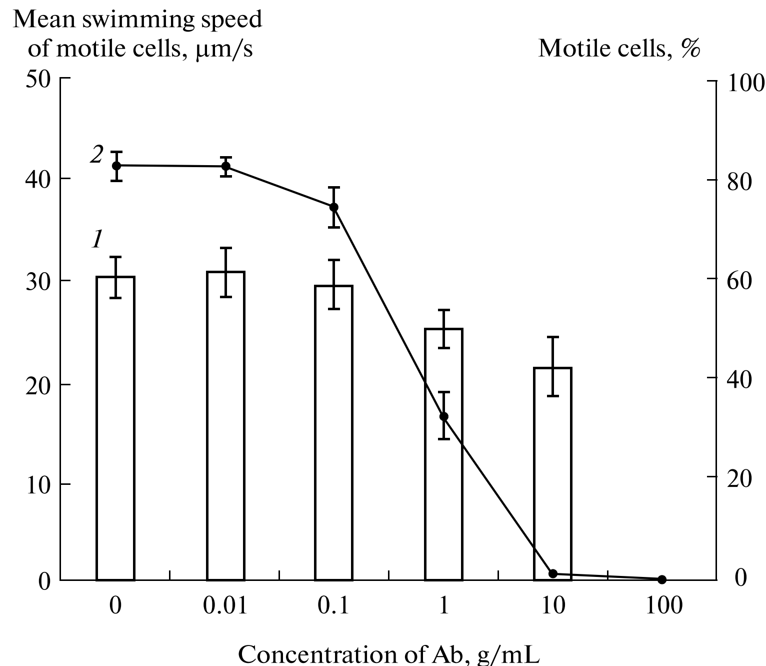

Fig. 2. Variation of the average rate of movement (*1*) and the number of motile *A. brasilense* Sp245 cells (*2*) at increasing concentrations of antibodies against the lipopolysaccharide of this strain.

Table 2. Motility of *A. brasilense* cells in the presence of antibodies against the lipopolysaccharides of strains Sp7 and Sp245

Strain	*Azospirillum* motility in PSB					
	without Ab (control)		with Ab_{Sp245}, 10 μg/mL		with Ab_{Sp245}, 100 μg/mL	
	motile cells, %	motion rate, μm/s	motile cells, %	motion rate, μm/s	motile cells, %	motion rate, μm/s
Sp7	85.5 ± 2.2	36.7 ± 2.8	84.0 ± 3.3	36.0 ± 3.7	3.0 ± 0.8	21.0 ± 1.7
Cd	87.3 ± 2.6	34.6 ± 3.1	80.4 ± 3.2	35.9 ± 3.4	21.0 ± 1.7	26.0 ± 4.5
SR55	58.3 ± 9.4	27.1 ± 2.4	40.6 ± 8.2	24.3 ± 4.1	7.1 ± 2.1	20.3 ± 1.7
SR80	51.6 ± 9.0	23.1 ± 2.4	51.3 ± 3.4	23.6 ± 2.2	9.5 ± 2.4	15.2 ± 3.1
Sp245	85.8 ± 3.8	29.9 ± 3.5	1.1 ± 0.9	21.4 ± 2.9	82.5 ± 2.2	32.7 ± 1.8
S27	85.2 ± 1.0	30.6 ± 2.0	6.2 ± 3.9	13.7 ± 4.8	82.0 ± 2.0	29.6 ± 3.6
SR15	86.6 ± 3.8	27.8 ± 3.4	5.0 ± 0.9	17.6 ± 2.6	83.9 ± 3.3	28.2 ± 3.9
SR75	60.9 ± 5.4	30.3 ± 3.3	11.4 ± 2.7	23.6 ± 1.9	54.1 ± 4.9	27.3 ± 3.6
Sp107	84.0 ± 2.8	31.3 ± 5.2	81.1 ± 2.9	28.1 ± 2.1	84.1 ± 4.8	33.6 ± 4.5
SR8	85.2 ± 2.2	30.5 ± 3.4	84.3 ± 1.8	29.7 ± 2.9	85.0 ± 1.6	30.5 ± 3.2

PSB, phosphate–saline buffer; Ab, antibodies; LPS, lipopolysaccharide.

flagellum (Fla) at one pole of the cell, which is used by the bacteria to move rectilinearly with random changes in the direction of motion. Figure 1 shows the motion trajectories of *A. brasilense* Sp245 cells, which is also typical of other *Azospirillum* strains used in the work (Table 1).

Addition of homologous anti-LPS Ab at a certain concentration to the *A. brasilence* Sp7 and Sp245 cell suspension had a negative effect on the number of motile cells and the average rate of their movement (see, e.g., Fig. 2). In the presence of anti-LPS Ab, motile bacteria visually showed the abrupt stops of freely floating cells or transition from translational to flip-flap motion. The minimum concentrations of strain-specific Ab considerably reducing motility indices for the strains Sp7 and Sp245 were 100 and 10 μg/mL, respectively (Fig. 2, Table 2). In case of the strain Sp7, the number of motile cells dropped from 85.5 ± 2.2% in the control to 3.0 ± 0.8% in the presence of 100 μg/mL of strain-specific Ab, while their motion rate decreased from 36.7 ± 2.8 to 21.0 ± 1.7 μm/s (Table 2). The number of motile cells of the strain Sp245 in the presence of 10 μg/mL of strain-specific Ab dropped from 85.8 ± 3.8 to 1.1 ± 0.9%, while the motion rate of swimming bacteria decreased from 29.9 ± 3.5 to 21.4 ± 2.9 μm/s (Fig. 2). When the concentration of Ab against Sp245 LPS increased to 100 μg/mL, bacterial motion stopped completely (Fig. 2). For *A. brasilense* Sp7, the concentration of anti-LPS Ab arresting cell motion was 1000 μg/mL. There was no cross impact of anti-LPS Ab on motility indices in the serologically different strains Sp7 and Sp245 [2] (Tables 2, 3).

The latter fact was the starting point for elaboration of a serological test procedure based on the inhibition of bacterial motility upon addition of strain-specific anti-LPS Ab to the cell suspension. The interaction between Ab and bacteria in this test was assessed by the motility of all cells in the field of view of the microscope and by the average motion rate of randomly selected motile cells. Cell aggregates appearing as a result of Ab interaction with corpuscular antigens were not taken into consideration. It should be noted that some bacterial cells (less than 10%) in liquid media form aggregates irrespective of the presence of antibodies in the medium. Aggregation is typical of azospirilla and may intensify due to the influence of some external factors on a bacterial population [21].

The series of experiments for studying cell motility in ten *Azospirillum* strains showed that the motility of some of them decreased in the presence of the Ab against Sp7 and Sp245 LPS at a concentration of 100 and 10 μg/mL, respectively (i.e., at Ab concentrations that caused a considerable decrease in motility indices of the homologous strains) (Table 2). However, cell motility of the strain Sp107 from serogroup I [2, 3] changed insignificantly in the presence of 10 μg/mL of Ab against Sp245 LPS (Table 2). The immunodiffusion test of LPS of two closely related *A. brasilense* strains, Sp245 and Sp107 [2, 3] demonstrated minor antigen differences manifesting themselves in the formation of a small spur in case of coalescence of immunoprecipitation bands [10]. This spur demonstrates that OPSI of the strain Sp107 lacks the antigen determinants that are present in OPSI of the strain Sp245. It is quite probable that this difference accounts for the insignificant decrease in motility of Sp107 cells in the presence of 10 μg/mL of Ab against the LPS of the closely related strain Sp245 (Table 2). The inhibitory effect was achieved by enhancing the concentration of Ab against Sp245 LPS to 1000 μg/mL, which

Table 3. Results of the interaction of antibodies against the LPS of *Azospirillum* strains of serogroups I and II with the cells and lipopolysaccharide extracts

Strain	Stoppage of cell motion by Ab		Cell agglutination by Ab, Ab titer		Immunoprecipitation of LPS extracts	
	Ab_{Sp7}	Ab_{Sp245}	Ab_{Sp7}	Ab_{Sp245}	Ab_{Sp7}	Ab_{Sp245}
A. brasilense:						
Sp7	+	–	1 :128	–	(++)	–
Cd	+	–	1 : 256	–	(++)	–
SR14	+	–	1 : 32	–	(+)	–
SR55	+	–	1 : 32	–	(++)	–
SR80	+	–	1 : 128	–	(+)	–
Sp245	–	+	–	1 : 512	–	(++)
Sp107	–	+	–	1 : 256	–	(++)
S27	–	+	–	1 : 256	–	(++)
SR15	–	+	–	1 : 256	–	(++)
SR75	–	+	–	1 : 512	–	(++)
SR8	–	–	–	–	–	–
SR32	–	–	–	–	–	–
SR41	–	–	–	–	–	–
SR50	–	–	–	–	–	–
SR64	–	–	–	–	–	–
SR72	–	–	–	–	–	–
SR87	–	–	–	–	–	–
SR88	–	–	–	–	–	–
Azospirillum sp.						
SR81	–	+	–	1 : 256	–	(++)
A. lipoferum:						
Sp59b	–	–	–	–	–	–
RG20a	–	+	–	1 : 128	–	(+)
A. irakense:						
KBC1	–	–	–	–	–	–
KA3	–	–	–	–	–	–

Ab, antibodies; LPS, lipopolysaccharides; (+) stoppage of cell motion; (++)/(+) two/one precipitation band(s) in the immunodiffusion reaction.

increased the number of Ab against the determinants common for Sp245 and Sp107 (Table 3).

Visualization of colloidal gold-labeled anti-LPS antibodies on bacterial surface. Electron microscopy of the cells labeled with anti-LPS Ab conjugated with colloidal gold showed the interaction between Ab and the surface, not only of the cell, but also of the polar flagellum (sheath) (Fig. 3). Both the cells with polar flagella densely covered with the label and the bacteria with less Ab-saturated flagella occurred (Fig. 3). The latter fact may account for the presence of the cells that remained motile in the presence of Ab. The maximum saturation of the cell and flagellum surface with antibodies may be not the only factor responsible for bacterial immobilization. Ab contributed to adhesion of the flagellum filament to bacterial cell surface (Fig. 3).

Thus, the results of microscopy showed that the decreased percentage of motile cells and the lower rate of their movement probably resulted from the saturation of bacterial surface with anti-LPS Ab, because the cell body or flagellum became heavier due to the inter-

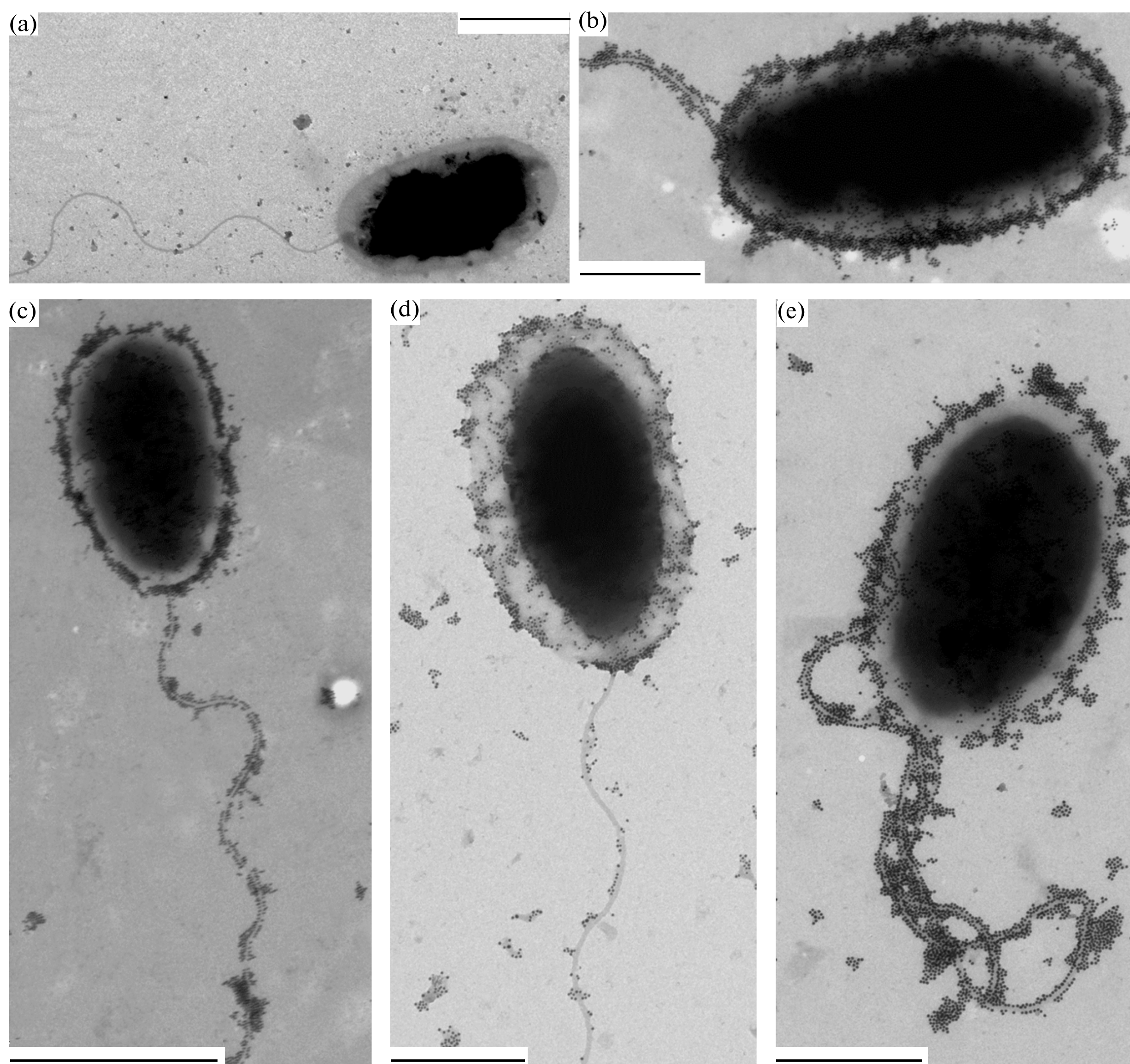

Fig. 3. Electron microphotographs of *A. brasilense* Sp245 cells (from 18-h cultures in liquid MSM) before (a) and after (b–e) their labeling with the antibodies conjugated with colloidal gold. Scale bar is 1 μm.

action with Ab. It is also probable that the Fla filament adheres to the bacterial body or to the Fla filament of another cell.

Interaction of antibodies against LPS of *A. brasilense* strains Sp245 and Sp7 from serogroups I and II with the cells and LPS extracts of other *Azospirillum* strains. The antibodies against the LPS of Sp7 (serogroup II) or Sp245 (serogroup I) halted only the motion of bacterial strains with the cells agglutinated by the respective Ab (Table 3). A correlation between the presence of antigenic chiasms in the immunodiffusion reaction and the decrease in cell motility in the presence of the Ab against Sp7 or Sp245 LPS in the strains Sp7, Cd, SR14, SR55 and SR80, or Sp245, Sp107, S27, SR15, SR75, SR81 and SpRG20a, respectively, was also revealed (Table 3).

In regard to 7 strains interacting with the Ab against Sp245 LPS, structural identity of the repeating unit of the OPS, which proved to be a linear penta-D-rhamnan, was established for *A. brasilense* Sp245, Sp107, S27, SR75, and *A. lipoferum* RG20a [2, 3]. While the OPS of SR15 is also a D-rhamnan, it has a tetrasaccharide repeating unit [3]. Our data make it possible to supplement serogroup I [2, 3] with the strain *Azospirillum* sp. SR81. *Azospirillum* strains of serogroup II possess heteropolysaccharide OPS cross-reacting with Ab against the LPS of *A. brasilense* Sp7 [2, 5]. The data on the structure of the repeating unit of OPS obtained for the three out of five *Azospirillum* strains (*A. brasilense* Cd, *A. irakense* KBC1, and *A. lipoferum* Sp59b interacting with the Ab against Sp7 LPS [2, 5]) account for their serological relationship with

A. brasilense Sp7. The results of this work allowed us to supplement serogroup II with the strain *A. brasilense* SR14.

It should be noted that the results of Ab influence on bacterial motility may be observed as early as 1 min after preparing the samples for microscopy (see Materials and Methods). The cells of the strains motile in the presence of Ab continued to move even after 30–60 min of incubation with the antibodies. The character of the effect of anti-LPS Ab on cell motility did not depend on the method of preparing bacterial suspensions. For example, the effects of Ab on the motility of *Azospirillum* cells in the cultures not washed from the cultivation medium or washed and suspended in PSB were not different.

Thus, the serological test consisting in the inhibition of bacterial motility by addition of anti-LPS Ab to the cell suspension is informative for the identification of the strains that demonstrate antigenic chiasms with the strains Sp7 and Sp245, which belong to different serological groups (serogroup II and serogroup I, respectively). The results of Ab influence on cell motility are in agreement with the data of such tests as Ab cell agglutination and detection of the presence of antigenic chiasms in the immunodiffusion reaction.

The proposed technique for assessment of the serological relationships between bacteria by the inhibition of their motility with anti-LPS antibodies includes determination of Ab concentrations negatively affecting the cell motion of the "model" strain, the relationship to which has yet to be established, and cultivation of the tested bacterial cultures, as well as preparation of the samples for light microscopy and assessment of bacterial motility in the samples. The strains with the cells immobilized in the presence of the Ab against the LPS of the "model" strain will be serologically close. The advantage of this technique is the rapid and simple detection of microbes with the common antigenic determinants in the structure of LPS, the predominant outer membrane components of gram-negative bacteria usually determining their serological specificity, which is essential for the immunotaxonomic studies of azospirilla.

REFERENCES

1. Fibach-Paldi, S., Burdman, S., and Okon, Y., Key physiological properties contributing to rhizosphere adaptation and plant growth promotion abilities of *Azospirillum brasilense, FEMS Microbiol. Lett.*, 2012, vol. 326, no. 1, pp. 99–108.
2. Konnova, O.N., Boiko, A.S., Burygin, G.L., Fedonenko, Yu.P., Matora, L.Yu., Konnova, S.A., and Ignatov, V.V., Chemical and serological studies of lipopolysaccharides of bacteria of the genus *Azospirillum, Microbiology* (Moscow), 2008, vol. 77, no. 3, pp. 305–312.
3. Boiko, A.S., Smol'kina, O.N., Fedonenko, Yu.P., Zdorovenko, E.L., Kachala, V.V., Konnova, S.A., and Ignatov, V.V., O-Polysaccharide structure in serogroup I azospirilla, *Microbiology* (Moscow), 2010, vol. 79, no. 2, pp. 197–205.
4. Fedonenko, Yu.P., Boiko, A.S., Zdorovenko, E. L., Konnova, S.A., Shashkov, A.S., Ignatov, V.V., and Knirel, Yu.A., Structural peculiarities of the O-specific polysaccharides of *Azospirillum* bacteria of serogroup III, *Biochemistry* (Moscow), 2011, vol. 76, no. 7, pp. 797–802.
5. Konnova, O.N., Burygin, G.L., Fedonenko, Yu.P., Matora, L.Yu., Pankin, K.E., Konnova, S.A., and Ignatov, V.V., Chemical composition and immunochemical characteristics of the lipopolysaccharide of nitrogen-fixing rhizobacterium *Azospirillum brasilense* Cd, *Microbiology* (Moscow), 2006, vol. 75, no. 3, pp. 323–328.
6. Matora L.Yu. and Shchegolev, S.Yu., Antigenic identity of the capsule lipopolysaccharides, exopolysaccharides, and O-specific polysaccharides in *Azospirillum brasilense, Microbiology* (Moscow), 2002, vol. 71, no. 2, pp. 178–181.
7. Croes, C.L., Moens, S., van Bastelaere, E., Vanderleyden, J., and Michiels, K., The polar flagellum mediated *Azospirillum brasilense* adsorption to wheat roots, *J. Gen. Microbiol.*, 1993, vol. 139, no. 9, pp. 2261–2269.
8. Doig, P. and Trust, T.J., Identification of surface-exposed outer membrane antigens of *Helicobacter pylori, Infect. Immun.,* 1994, vol. 62, no. 10, pp. 4526–4533.
9. Fuerst, J.A. and Perry, J.W., Demonstration of lipopolysaccharide on sheathed flagella of *Vibrio cholerae* O:1 by protein A-gold immunoelectron microscopy, *J. Bacteriol.*, 1988, vol. 170, no. 4, pp. 1488–1494.
10. Burygin, G.L., Shirokov, A.A., Shelud'ko, A.V., Katsy, E.I., Shchygolev, S.Yu., and Matora, L.Yu., Detection of a sheath on *Azospirillum brasilense* polar flagellum, *Microbiology* (Moscow), 2007, vol. 76, no. 6, p. 728–734.
11. Eskew, D.L., Focht, D.D., and Ting, I.P., Nitrogen fixation, denitrification, and pleomorphic growth in a highly pigmented *Spirillum lipoferum, Appl. Environ. Microbiol.*, 1977, vol. 34, no. 5, p. 582–585.
12. Tarrand, J.J., Krieg, N.R., and Döbereiner, J., A taxonomic study of the *Spirillum lipoferum* group with description of a new genus, *Azospirillum* gen. nov. and two species, *Azospirillum lipoferum* (Beijerinck) comb. nov. and *Azospirillum braslense* sp. nov., *Can. J. Microbiol.*, 1978, vol. 24, no. 8, p. 967–980.
13. De-Polli, H., Bohlool, B.B., and Döbereiner, J., Serological differentiation of *Azospirillum* species belonging to different host-plant specificity groups, *Arch. Microbiol.*, 1980, vol. 126, no. 3, p. 217–222.
14. Baldani, V.L.D., Baldani, J.I., and Döbereiner, J., Effects of *Azospirillum* inoculation on root infection and nitrogen incorporation in wheat, *Can. J. Microbiol.*, 1983, vol. 29, no. 8, p. 924–929.
15. Pozdnyakova, L.I., Kanevskaya, S.V., Levanova, G.F., Barysheva, N.N., Pilipenko, T.Yu., Bogatyrev, V.A., and Fedorova, L.S., Taxonomic studies of *Azospirillum* species isolated from cereals of Saratov oblast, *Mikrobiologiya*, 1988, vol. 57, no. 2, pp. 222–225.

16. Khammas, K.M., Ageron, E., Grimont, P.A., and Kaiser, P., *Azospirillum irakense* sp. nov., a nitrogen-fixing bacterium associated with rice roots and rhizosphere, *Res. Microbiol.*, 1989, vol. 140, no. 9, pp. 679–693.

17. Döbereiner, J. and Day, J.M., Associative symbiosis in tropical grass: characterization of microorganisms and dinitrogen fixing sites, in *Symposium on Nitrogen Fixation*, Newton, W.E. and Nijmans, C.J., Eds., Pullman: Washington State Univ. Press, 1976, pp. 518–538.

18. Matora, L.Yu., Shvartsburd, B.I., and Shchegolev, S.Yu., Immunochemical analysis of O-specific polysaccharides from the soil nitrogen-fixing bacteria *Azospirillum brasilense, Microbiology* (Moscow), 1998, vol. 67, no. 6, pp. 677–681.

19. Dykman, L.A. and Bogatyrev, V.A., Gold nanoparticles: preparation, functionalization and applications in biochemistry and immunochemistry, *Russ. Chem. Rev.*, 2007, vol. 76, no. 2, pp. 181–205.

20. Shelud'ko, A.V., Makrushin, K.V., Tugarova, A.V., Krestinenko, V.A., Panasenko, V.I., Antonyuk, L.P., and Katsy, E.I., Changes in motility of the rhizobacterium *Azospirillum brasilense* in the presence of plant lectins, *Microbiol. Res.*, 2009, vol. 164, no. 2, pp. 149–156.

21. Burdman, S., Jurkevitch, E., Schwartsburd, B., Hampel, M., and Okon, Y., Aggregation in *Azospirillum brasilense*: effects of chemical and physical factors and involvement of extracellular components, *Microbiology* (UK), 1998, vol. 144, no. 7, pp. 1989–1999.

Translated by E. Makeeva