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EXPERIMENTAL ARTICLES

Antigenic Identity of the Capsule Lipopolysaccharides, Exopolysaccharides, and O-Specific Polysaccharides in *Azospirillum brasilense*

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Abstract—The antigenic identity (and close values of electrophoretic mobility) of capsular polysaccharides, exopolysaccharides, and O-specific polysaccharides was revealed in the *Azospirillum brasilense* strains Sp7 and Sp245 by the immunodiffusion and immunoelectrophoretic methods. Together with the literature data on the identity of the monosaccharide composition of these polymers, this gives evidence of the absence of a specific capsular antigen in the bacteria studied. Thus, extracellular *Azospirillum brasilense* polysaccharides are likely to represent O-antigenic lipopolysaccharide fragments excreted by the bacteria into the culture medium, and their identification as a capsule or as an exopolysaccharide depends on the strength of the attachment of these polysaccharides to the cell surface.

Key words: azospirilla, lipopolysaccharides, exopolysaccharides, capsular polysaccharides.

Lipopolysaccharide (LPS) is the main component of the outer membrane of gram-negative bacteria. The molecules of LPS include three regions different in their composition and structure: the hydrophobic part (lipid A), binding LPS to the cell membrane; the oligosaccharide region (referred to as the core); and the polysaccharide chain (O-specific polysaccharide (O-PS)), connected with lipid A via the core [1]. LPS determine the immunogenic properties of bacteria [2]; therefore, they are often referred to as somatic antigens or O-antigens (O-Ag).

In addition to LPS, extracellular polysaccharides are envelope components of gram-negative bacteria. They are disposed above the outer membrane of the bacterial cell and can exist in the form of a capsule or free mucus. In a number of cases, capsular polysaccharides (CPS), exopolysaccharides (EPS), and LPS appear to be identical in their chemical and antigenic structure [3]. In particular, it was shown that O-Ag and capsular antigens (C-Ag) do not differ chemically in *Escherichia coli* [4]. The oligosaccharide C-Ag chains can attach to the cell surface by means of the core-lipid A (the so called C-LPS) [5], and their distinction mainly reflects their contribution to serological specificity, because longer C-Ag mask shorter O-Ag. Moreover, in serological studies of *Escherichia coli*, the same polysaccharides may be classified as O-Ag in one strain and as C-Ag in other strains [6]. It is possible that this phenomenon is not unique to pathogenic bacteria and occurs in other groups of microorganisms as well.

Associative nitrogen-fixing soil bacteria of the genus *Azospirillum* are assigned to gram-negative microorganisms and contain LPS as the main constituent of their external membrane. In addition, azospirilla were shown to be capable of synthesizing CPS and EPS [7, 8]. Ital'yanskaya *et al.* [9] revealed the presence of common antigenic determinants in the CPS and LPS of *A. brasilense* Sp7, and Konnova *et al.* [10] showed that the monosaccharide compositions of the polysaccharide-containing complexes isolated from the culture fluid and cell surface of azospirilla were identical.

In this work, we made a detailed comparison of the antigenic properties of LPS and their O-PS with the antigenic properties of the capsular polysaccharides and exopolysaccharides of the *A. brasilense* strains Sp7 and Sp245.

MATERIALS AND METHODS

The strains studied are listed in the table. The bacteria were grown on a shaker until the late logarithmic phase in liquid synthetic malate medium [14]. Polyclonal strain-specific antibodies (Ab) against azospirillum LPS were obtained as described in [15]. LPS were obtained by the method proposed by Leive *et al.* [16]. The CPS and EPS preparations were kindly provided by D.A. Zhemerichkin and S.A. Konnova. Double immunodiffusion was performed according to Ouchterloni and Nilsson [17]. Line, crossed, tandem, and fused rocket immunophoresis was carried out according to Axelson *et al.* [18]. Agarose gels (1%) prepared on

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Tris–glycine–barbituric buffer (ionic strength 0.02, pH 8.8) were used for immunodiffusion and immunoelectrophoresis. Line electrophoresis was performed for 30 min at 10 V/cm. On the completion of electrophoresis, 100 μl of Ab (at a concentration of 40 mg/ml) was introduced into each pit. For crossed immunophoresis, the samples were introduced into the first direction gel wells (without Ab). In the first direction, electrophoresis was carried out for 30 min at 10 V/cm; in the second direction, for 16 h at 2 V/cm. The second direction gel contained 1% of the corresponding Ab. Before tandem crossed immunoelectrophoresis, the plate with the first direction gel was allowed, upon introduction of the samples, to stand for 30 min in a humid chamber for the preparations to diffuse into the gel. Electrophoresis was further performed in the same fashion as crossed electrophoresis. For fused rocket immunoelectrophoresis, a combined gel was prepared: one portion of the gel (with the wells for samples) did not contain Ab, and the second portion contained 1% Ab. In this case, the wells were disposed one above the other and had different shapes. LPS preparations were introduced into oblong wells; CPS, into round wells. The plate with the gel was allowed to stand in the humid chamber for the preparations to diffuse into the gel with subsequent low-voltage immunoelectrophoresis (16 h, 2 V/cm). After squeezing and multiple washing procedures, the gels were stained with Coomassie R-250.

RESULTS AND DISCUSSION

Figure 1 shows the results of immunodiffusion analysis of the LPS, CPS, and EPS preparations of strain Sp7 with Ab against the LPS of this strain. The LPS preparation formed two precipitation lines with Ab, which attests to the presence in the preparation of two polymer fractions that differ in antigenic properties. This agrees well with our earlier obtained data showing that the LPS of *A. brasilense* Sp7 contains two O-PS [19]. As can be seen from Fig. 1, the CPS and EPS preparations formed fused precipitation bands with one of the two O-PS entering the composition of the LPS of this strain.

For a more complete comparison of the antigenic composition of the studied components, we used a combination of different immunochemical methods. The results of line immunoelectrophoresis (Fig. 2) show that the antigenic determinants of all three preparations have close values of electrophoretic mobility. Figure 3 demonstrates the results of (a) crossed immunoelectrophoresis of the LPS preparation, (b) tandem crossed phoresis of LPS and EPS, and (c) fused rocket phoresis of LPS and CPS. It can be seen that CPS and EPS form precipitation peaks common with LPS, without any additional peak formation. The results obtained allow the conclusion to be made that the LPS of this strain has immunodeterminant sites similar to those in both CPS and EPS. Taking into account that, according to the data of Konnova *et al*. [10], the above polymers have the same monosaccharide composition, we can speak about the absence of a specific capsular material in strain Sp7. In this case, the capsule and EPS seem to represent O-specific LPS fragments excreted by the cell into the surroundings.

Table

Azospirillum brasilense strains	Characterization	Reference
Sp7	Wild type	[11]
Sp245	Wild type	[12]
KM018	Omegon mutant of strain Sp245 with insertion of omegon-KM in the 120-MDa plasmid	[13]

We obtained similar results when studying the carbohydrate-containing components of the cell surface of another strain—*A. brasilense* Sp245. Earlier, we also established the presence of two O-specific polysaccharides in the LPS composition of this strain: O-PS1 and O-PS2 [13]. In addition, in this work, we also studied KM018, a mutant of strain Sp245, in which only one antigen, O-PS1, is present as a LPS constituent (table). Figure 4 shows the result of immunodiffusion analysis

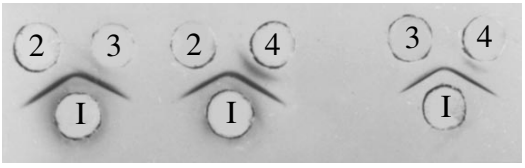

Fig. 1. Double immunodiffusion of the CPS (2), EPS (3), and LPS (4) preparations of *A. brasilense* Sp7 with the antibodies against the LPS of this strain (1).

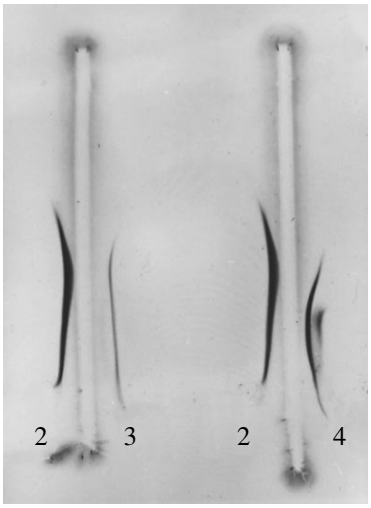

Fig. 2. Line immunoelectrophoresis of the CPS (2), EPS (3), and LPS (4) preparations of *A. brasilense* Sp7 with the antibodies against the LPS of this strain.

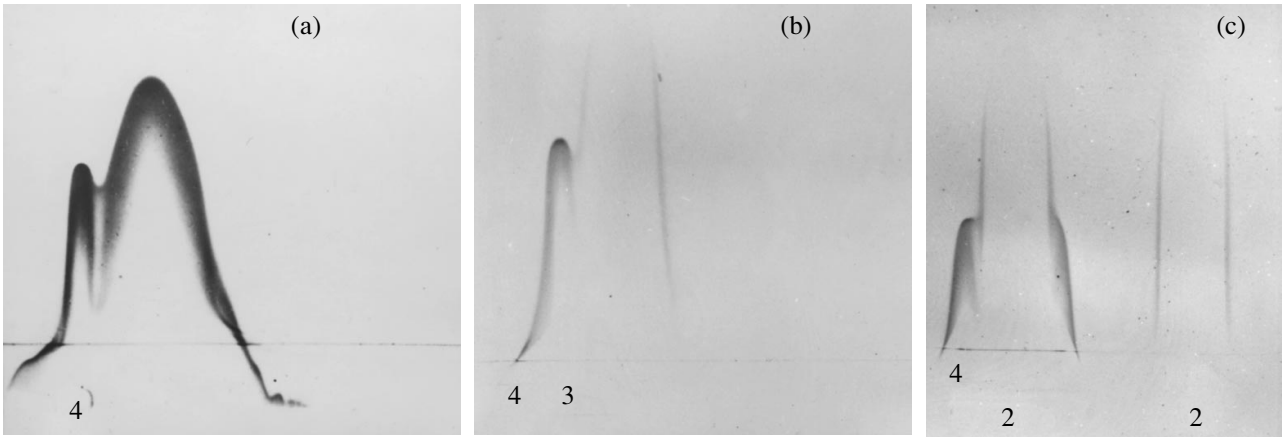

Fig. 3. Crossed immunoelectrophoresis (a), tandem crossed immunoelectrophoresis (b), and fused rocket immunoelectrophoresis (c) of the CPS (2), EPS (3), and LPS (4) preparations with the antibodies against the LPS of *A. brasilense* Sp7.

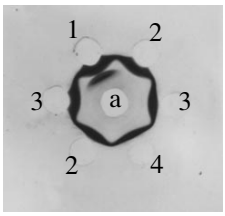

Fig. 4. Double immunodiffusion of the LPS (1), O-PS1 (2), EPS (3), and CPS (4) preparations of *A. brasilense* Sp245 with the antibodies against the LPS of this strain (a).

of the LPS, CPS, and EPS preparations of strain Sp245 and the LPS preparation of KM018. It should be noted that, in this case, CPS and EPS designate the polysaccharide–lipid complexes isolated by Konnova *et al.* [10], respectively, from the capsule and culture fluid of strain Sp245. The fusion of precipitation bands formed by CPS, EPS, and O-PS1 gives evidence of the complete identity of their antigenic determinants; i.e., the capsule and EPS of strain Sp245 consist of a substance that contains antigenic determinants identical to those in O-PS1. Thus, of the two O-PS revealed in the LPS composition of strain Sp245, it is O-PS1 that evidently forms the capsule substance and the EPS of *A. brasilense* Sp245.

In our opinion, the results obtained in this work lead one to refine the currently existing notions of the independent role of the EPS of the bacteria studied in the establishment of associative interrelations between azospirilla and plants and may be used for the design of specific probes for taxonomic and ecological studies of azospirilla.

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