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EXPERIMENTAL ARTICLES

Investigation of Lipopolysaccharides from *Sinorhizobium meliloti* SKHM1-188 and Two of Its Mutants with Decreased Nodulation Competitiveness

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Abstract—A comparative study of the lipopolysaccharides (LPS) isolated from *Sinorhizobium meliloti* SKHM1-188 and two of its LPS mutants (Tb29 and Ts22) with sharply decreased nodulation competitiveness was conducted. Polyacrylamide gel electrophoresis with sodium dodecyl sulfate revealed two forms of LPS in all three strains: a higher molecular weight LPS1, containing O-polysaccharide (O-PS), and a lower molecular weight LPS2, without O-PS. However, the LPS1 content in mutants was significantly smaller than in the parent strain. The LPS of the strains studied contained glucose, galactose, mannose, xylose, three nonidentified sugars (X_1 (TGlc 0.53), X_2 (TGlc 0.47), and X_3 (TGlc 0.43)), glucosamine, and ethanolamine, while the LPS of *S. meliloti* SKHM1-188 additionally contained galactosamine, glucuronic and galacturonic acids, and 2-keto-3-deoxyoctulosonic acid (KDO), as well as such fatty acids as 3-OH C14:0, 3-OH C15:0, 3-OH C16:0, 3-OH C18:0, nonidentified hydroxy *X* ($T_{3\text{-OH C14:0}}$ 1.33), C18:0, and unsaturated C18:1 fatty acids. The LPS of both mutants were similar in the component composition but differed from the LPS of the parent strain by lower X_2, X_3, and 3-OH C14:0 contents and higher KDO, C18:0, and hydroxy *X* contents. The LPS of all the strains were subjected to mild hydrolysis with 1% acetic acid and fractionated on a column with Sephadex G-25. The higher molecular weight fractions (2500–4000 Da) contained a set of sugars typical of intact LPS and, supposedly, corresponded to the LPS polysaccharide portion (PS1). In the lower molecular weight fractions (600–770 Da, PS2), glucose and uronic acids were the major components; galactose, mannose, and X_1 were present in smaller amounts. The PS1/PS2 ratio for the two mutants was significantly lower than for strain SKHM1-188. The data obtained show that the amount of O-PS–containing molecules (LPS1) in the heterogeneous lipopolysaccharide complex of the mutants was smaller than in the SKHM1-188 LPS; this increases the hydrophobicity of the cell surface of the mutant bacteria, which supposedly contributes to their nonspecific adhesion to the roots of the host plant, thus decreasing their nodulation competitiveness.

Key words: *Sinorhizobium meliloti*, legume–*Rhizobium* symbiosis, lipopolysaccharide, nodulation competitiveness.

Glycopolymers, in particular lipopolysaccharides (LPS), play an important role in the complex and multistage process of the establishment of legume–*Rhizobium* symbiosis [1]. Being a component of the regulatory mechanism of lectin–carbohydrate recognition, LPS determine the specificity and, therefore, effectiveness of the nitrogen-fixing symbiotrophic system. In addition to performing various biological effector functions, lipopolysaccharides, owing to the presence of the lipid portion in their composition, make a contribution to the hydrophobicity of the cell surface of bacteria. This affects the nonspecific adhesion of rhizobia on plants since the surface of plant cells is also hydrophobic. Thus, not only the compatibility of rhizobia with the host plant but also their infectious properties—virulence and competitiveness—may depend on the physicochemical features and macrostructural organization of LPS [2, 3].

In the fight between strains for nodule formation on the roots of leguminous plants, nodulation competitiveness, as well as specificity and virulence, is a factor favoring the preservation of strain specialization of rhizobia with respect to one or another host plant. On the other hand, in the fight of rhizobia introduced as components of bacterial fertilizers with the aboriginal microflora for root nodule formation on the host plant, the nodulation competitiveness of the introduced strains plays a decisive role and, in the final analysis, determines the expediency of their use.

Earlier, we obtained, on the basis of strain *Sinorhizobium meliloti* SKHM1-188, two Tn5 mutants (Tb29 and Ts22) that synthesize, as judged from the

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data of polyacrylamide gel electrophoresis with sodium dodecyl sulfate, a modified LPS. Both strains displayed unchanged efficiency and nitrogen-fixing activity but exhibited dramatically decreased competitiveness [4, 5].

We established that the genome of each of the mutants contains a single insertion of transposon Tn5 [5]. Thus, a single damage to one of the numerous genes involved in LPS synthesis resulted in a decrease in the rhizobial competitiveness, which indicates the dependence of the symbiotic status of rhizobia on the properties of their LPS.

In this connection, the aim of this work was to conduct a comparative study and to reveal the differences between the lipopolysaccharides of the *S. meliloti* parent strain and two of its LPS mutants characterized by sharply decreased nodulation competitiveness.

MATERIALS AND METHODS

Bacteria. Three *Sinorhizobium* (formerly *Rhizobium*) *meliloti* strains (alfalfa rhizobia)—strain SKHM1-188 (the *Rhizobium* collection of the All-Russia Research Institute of Agricultural Microbiology, St. Petersburg–Pushkin) and two of its Tn5-induced mutants, Tb29 and Ts22—were used in this work [4]. The bacteria were grown in a liquid medium containing peptone (10 g/l), yeast extract (1 g/l), and $CaCl_2$ (0.2 g/l) at 28–30°C for 24 h.

Isolation of LPS. The bacterial cells were precipitated by centrifugation at 5500 *g* for 40 min, washed five times with saline, and dried with acetone and ethyl ether. LPS were extracted from dry rhizobial cells with a 45% water–phenol mixture according to Westphal and Jann's technique [6]. To remove acidic exoglycans and nucleic acids, the LPS were treated with 2% Cetavlon in 0.5 M NaCl. The mixture was centrifuged at 5500 *g* for 30 min. The LPS were precipitated from the supernatant fluid with a 10-fold volume of ethanol and dialyzed against distilled water. Ultracentrifugation at 105000 *g* (three times for 5 h) was carried out for the purification of the periplasm glucan, with which rhizobial LPS are generally associated. The LPS (precipitate) was dried lyophilically.

Polyacrylamide gel electrophoresis with sodium dodecyl sulfate was performed according to the standard method [7]. Both wet cells treated with proteinase K as described earlier in [4] and the isolated LPS preparations were analyzed. The gels were stained with silver nitrate and alcyanogen blue.

Partial LPS degradation. Mild acid hydrolysis of LPS was performed with 1% acetic acid (4 h, 100°C). The polysaccharide moiety (PS) was separated from lipid A by centrifugation at 5500 *g* for 30 min.

Lipid A (precipitate) was sequentially washed with 0.01 M HCl, methanol, and acetone to purify it of phospholipid impurities. The PS (supernatant fluid) was centrifuged at 25000 *g* to precipitate the nondegraded LPS residues. Drying was lyophilic.

The PS fractionation was carried out on a 55.4 × 2.1 cm column filled with Sephadex G-25 (Pharmacia) and equilibrated with pyridine–acetate buffer, pH 4.5. The volume of fractions was 3.0 ml; the buffer flow rate was 0.3 ml/min. The elution profiles were constructed from the contents of total carbohydrates, uronic acids, and 2-keto-3-deoxyoctulosonic acid (KDO) in the fractions. To calibrate the column and to determine the molecular mass (MM) of the LPS polysaccharide fractions, standard carbohydrates were used: glucose (180 Da), mannose (180 Da), glucoheptose (210 Da), trehalose (342 Da), raffinose · $5H_2O$ (594 Da), stachyose (666 Da), Blue Dextran (2 million Da) (Sigma), and Dextran-10 (9800 Da) (Pharmacia). The molecular mass was calculated according to Determan [8] proceeding from the linear dependence of v_e/v_0 on logMM, where v_e is the fraction volume eluted from the column and v_0 is the idle gel volume.

Study of the component composition. To determine neutral carbohydrates, preliminary hydrolysis of the LPS preparations with 2 N HCl was performed in sealed ampoules (6 h, 100°C). The hydrolysates obtained were analyzed by gas–liquid chromatography (GLC) on a Chrom-5 chromatograph (Czechoslovakia) equipped with a flame-ionization detector and a column (120 × 0.3 cm) with an SP 2340 stationary phase on Chromaton (100 mesh). The thermostat temperature was programed to rise from 170 to 230°C at a rate of 3°C/min; the injector temperature was 240°C. The helium and hydrogen flow rates were 30 cm^3/min; the flow rate of air was 300 cm^3/min. Sugars were identified from the retention time (*T*) of their polyol acetates and its comparison with that of the corresponding standards. For unidentified sugars, the retention times were established in relation to that of glucose (Tglc). The relative content of each component was calculated from the polyol acetate peak areas in the GLC chromatograms.

Fatty acids were determined after methanolysis of the LPS preparations with an anhydrous solution of 2 N HCl in methanol in sealed ampoules (4 h, 100°C). Methyl esters of fatty acids were extracted from the reaction mixture with hexane (three times by 3 ml) and determined by GLC on a Chrom-5 chromatograph (Czechoslovakia) using two columns. One column (120 × 0.3 cm) was filled with 5% SE-30 on Chromaton N-AW-DMCS (Lachema, Czechoslovakia); the temperature was programed to rise from 125 to 250°C at a rate of 3°C/ min. The other column (200 × 0.3 cm) was filled with 5% DEGS-PS on Supelcoport (100–120 mesh) (Supelco SA, Switzerland) and was operated at 200°C. The carrier gas was helium. Fatty acids were identified from their retention times compared with those of standards. Hydroxy fatty acids were identified from by GLC from the retention times after treatment of methyl esters of fatty acids with trifluoroacetic anhydride [9]; the GLC conditions were the same as specified above for methyl esters of fatty acids. For uni-

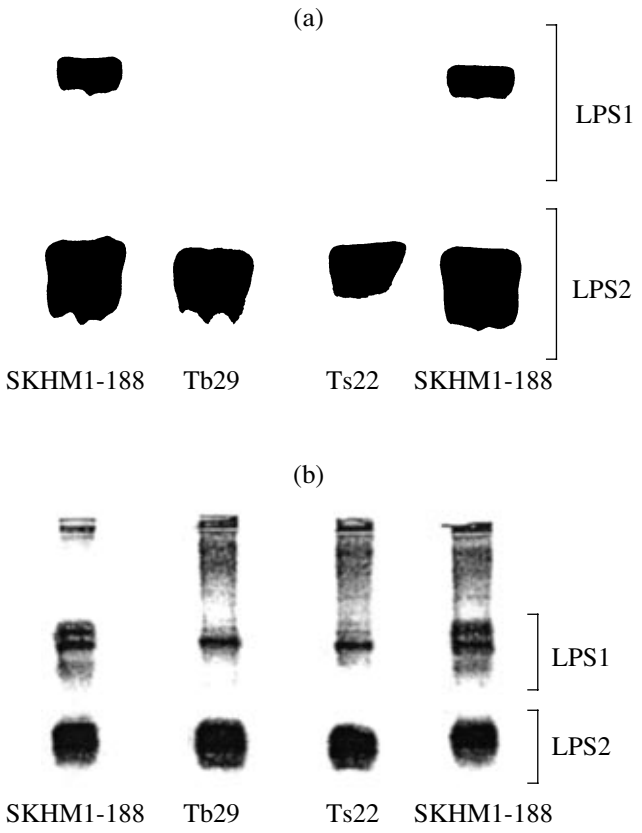

Fig. 1. Polyacrylamide gel electrophoresis of the LPS of *S. meliloti* SKHM1-188 and two of its Tn5-induced mutants, Tb29 and Ts22. Gel staining by (a) silver nitrate and (b) alcyanogen blue and then silver nitrate.

dentified hydroxy fatty acids, the retention times were calculated in relation to 3-OH C14:0 ($T_{3\text{-OH C14:0}}$).

The presence and content of amine compounds (glucosamine, galactosamine, ethanolamine) were determined using a Biotronik LS 5001 amino acid analyzer (Germany) after the hydrolysis of the LPS preparations and lipid A with 4 N HCl (18 h, 100°C) in sealed ampoules.

The determination of heptoses, KDO, and uronic acid was carried out using the conventional methods described in [10]. The total carbohydrate content in the LPS preparations was determined according to [11].

Table 1. Yield and general characteristics of LPS preparations from *S. meliloti*

Strain	LPS yield, % of dry cell mass	Carbohydrate content	Protein content
		% of dry preparation mass	
SKHM1-188	0.70	26.0	2.2
Tb29	0.18	39.2	1.8
Ts22	0.74	26.4	1.2

The protein content was determined by the method of Lowry *et al.* [12].

RESULTS AND DISCUSSION

The LPS molecule is known to consist of three main portions: lipid A and the polysaccharide portion, which is subdivided into the core and O-polysaccharide (O-PS), also called the O-antigen. The O-polysaccharide has a regular structure and consists of repetitive saccharide links. The full LPS form containing all three structural regions is designated as S-LPS, and LPS with a missing O-chain is designated as R-LPS. However, due to the specific features of the structural organization of rhizobial LPS (possibly, the absence of stringent regularity in the structure of the O-polysaccharide), they are designated as LPS1 (the full O-polysaccharide–containing LPS form) and LPS2 (containing only lipid A and the oligosaccharide core) [15, 17].

According to the data of polyacrylamide gel electrophoresis with sodium dodecyl sulfate (Figs. 1a, 1b), strains Tb29 and Ts22 obtained on the basis of *S. meliloti* SKHM1-188 were identified as LPS mutants. When the gels were stained with silver nitrate according to the standard technique, they either did not exhibit the LPS1 region [4] or it manifested itself so slightly that it could be taken for an artifact. However, when alcyanogen blue was used, the electrophoregrams exhibited a narrow region corresponding to the LPS1 region. These results, irrespective of the stain used, indicate that the mutants Tb29 and Ts22 have lost the ability to synthesize the full complex of the O-polysaccharide–containing LPS forms constituting the LPS1 region.

The LPS yield from the dry cells of all three strains studied was modest: 0.18–0.74% of their mass. The carbohydrate content in the LPS preparations constituted 26.0–39.2%, and that of proteins was 1.2–2.2% (Table 1).

The LPS studied had the same monosaccharide composition (Table 2). They all contained glucose (the dominant sugar), galactose, mannose, traces of xylose, three unidentified sugars (X_1 (Tglc 0.53, close to ribose in the retention time), X_2 (Tglc 0.47, supposedly deoxyhexose), and X_3 (Tglc 0.43, the retention time close to that of fucose and rhamnose)), glucuronic and galacturonic acids, the amine compounds glucosamine and ethanolamine (in addition, galactosamine was found in the LPS of SKHM1-188), and KDO.

At the same time, the LPS of the mutants differed from the LPS of SKHM1-188 and also between each other in the relative content of individual sugars. Thus, the LPS of strains Tb29 and Ts22 had a lesser amount of unidentified sugars X_2 (Tglc 0.47) and X_3 (Tglc 0.43) than the LPS of SKHM1-188.

This suggests that the above-mentioned sugars may be constituents of the O-polysaccharide, which in mutants is likely to have a reduced total size compared

to the LPS O-PS of SKHM1-188. Such a suggestion agrees with the data on the higher (by almost twofold) LPS content of KDO in both mutants compared with the LPS of the parent strain and the results of polyacrylamide gel electrophoresis with sodium dodecyl sulfate. As seen from Fig. 1b, the LPS1 region in both mutants, as judged by staining of the electrophoregrams with alcyanogen blue, is by far smaller than in the LPS of strain SKHM1-188.

The LPS fatty acid composition of all three strains was identical (Table 3). They contained four hydroxy fatty acids—3-OH C14:0 (the dominant component), 3-OH C15:0, 3-OH C16:0, and 3-OH C18:0, whose total content constituted 73–83% of the total content of fatty acids, as well as unidentified hydroxy *X* ($T_{3\text{-OH C14:0}}$ 1.33), saturated C18:0, and unsaturated C18:1 fatty acids. Unfortunately, we had no technical facilities to judge the presence or absence of the long-chain fatty acid 27 OH C28:O, typical of rhizobial LPS [13].

As in the case of the monosaccharide composition, the LPS of mutants not only differed in the relative fatty acid content from the LPS of SKHM1-188 but also between each other. The most characteristic feature of the LPS of both mutants compared to the parent strain LPS was their lesser 3-OH C14:0 content against the increase of two other fatty acids—C18:0 and hydroxy *X*. Between each other, the LPS of mutants differed in the relative 3-OH C15:0 content, which in the Tb29 LPS was twice as small as in the Ts22 LPS.

Despite the slight differences (the presence of galactosamine, ethanolamine, hydroxy *X*, etc.), the LPS of the strains studied had the component composition typical of the *S. meliloti* LPS [14–16].

To study the lipopolysaccharides in greater detail, their mild degradation with 1% acetic acid, which results in the cleavage of the bond between the LPS polysaccharide (PS) portion and lipid A, was carried out. Gel filtration on the column with Sephadex G-25 (Figs. 2–4) caused the partially degraded LPS polysaccharides of the strains studied to be separated into four (Tb29) or five (SKHM1-188, Ts22) fractions. As evidenced by the composition of the monosaccharides and their content (Tables 4–6), fractions I in the LPS mutants (approximately 4600–5000 Da) might be concomitant polysaccharides. This is indicated by the low content or the absence in them of, primarily, uronic acids, as well as KDO. Fraction I of strain SKHM1-188 (4000 Da) and fractions II of all the three strains (approximately 2500–2900 Da), taking into account that they contain all the sugars characteristic of nondegraded LPS, are likely to represent PS1 (i.e., the core with the O-PS). Evidently, fractions III of the three strains studied (600–770 Da) appear to be oligosaccharides formed from LPS2 (the core without the O-PS). The predominant component of these fractions in the bacteria studied is uronic acids and glucose. Galactose and X_1, as well as mannose and KDO, are present in them in lesser amounts. The fraction III content of galactose, X_1, and mannose, a large amount of which is present in the lowest molecular weight (180–350 Da) fractions IV and V—cleaved dimers of different composition and monomers—varies significantly.

Table 2. Monosaccharide composition of the LPS of *S. meliloti* SKHM1-188 and two of its LPS mutants, Tb29 and Ts22

Component	Strain		
	SKHM1-188	Tb29	Ts22
% of the total peak area in GLC chromatograms			
Glucose	54.9	61.0	57.9
Galactose	12.9	15.1	11.3
Mannose	6.8	2.9	5.5
Xylose	traces	traces	traces
X_1 (Tglc 0.53)	2.5	7.7	4.1
O_2 (Oglc 0.47)	8.4	5.1	10.3
X_3 (Tglc 0.43)	14.5	8.2	10.9
% of dry preparation mass			
Uronic acids	11.62	9.32	9.34
Glucosamine	2.44	1.85	2.45
Galactosamine	0.15	not detected	not detected
Ethanolamine	0.24	0.18	0.22
KDO	5.2	10.8	10.8

Table 3. Fatty acid composition of the LPS of *S. meliloti* SKHM1-188 and two of its LPS mutants, Tb29 and Ts22

Component	Strain		
	SKHM1-188	Tb29	Ts22
% of the total peak area in GLC chromatograms			
3-OH C14:0	48.5	41.0	38.5
3-OH C15:0	14.2	7.9	15.9
3-OH C16:0	5.6	4.6	6.7
3-OH C18:0	14.2	19.6	14.3
C18:0	3.7	11.6	8.7
C18:1	11.4	10.1	11.1
Hydroxy *X* ($T_{3\text{-OH C:14 1.33}}$)	2.4	5.2	4.8

The small molecular mass of fractions III and different ratios of individual sugars entering into their composition in different strains indicate that a mixture of low-molecular-weight core oligosaccharides was formed in the process of LPS degradation. Moreover, fragments from the O-PS may find their way into these fractions. The presence of labile bonds between the O-PS and the core, as well as inside these structural portions, was noted by other authors who studied the products of mild degradation of rhizobial LPS with acetic acid [14–17]. This lability is determined by the presence of KDO not only between lipid A and the core but also in other sites of the lipopolysaccharide molecule [1].

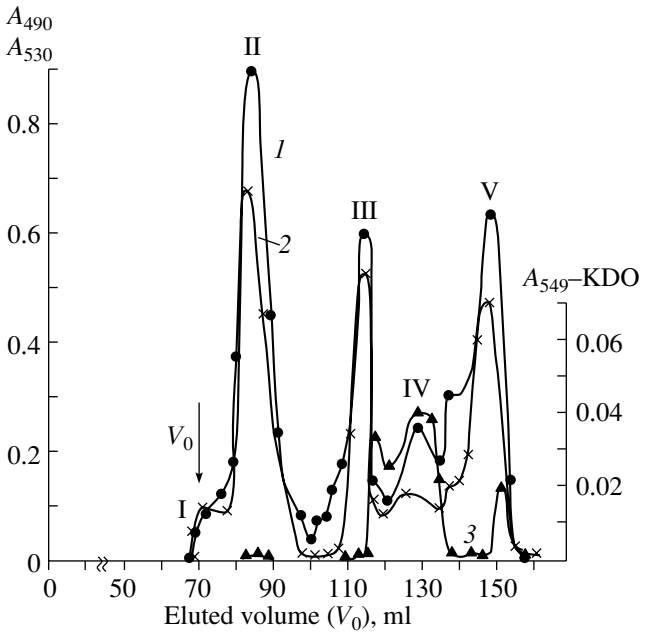

Fig. 2. Gel filtration on Sephadex G-25 of polysaccharides obtained as a result of partial degradation of *S. meliloti* SKHM1-188 LPS with 1% acetic acid. Here and in Figs. 3 and 4: (*1*) carbohydrates (A_{490}); (*2*) uronic acids (A_{530}); (*3*) KDO (A_{549}).

In lipid A of all the strains studied, the presence of glucosamine and ethanolamine was established at an approximate ratio of 10 : 1. Their sum constituted 7–9% of the weight of the preparation.

On the basis of the comparative analysis of the monosaccharide composition of nondegraded LPS and individual fractions of partially degraded LPS, we suggested that the core oligosaccharide composition may include glucose, uronic acids, and, possibly, galactose and X_1. We may assume with a high degree of probability that O-polysaccharide contains unidentified sugars, X_2 and X_3, and amino sugars (galactosamine) as its constituents.

The ratio of the fractions that contained the LPS1 and LPS2 oligosaccharide fragments (PS1/PS2) (Table 7) testifies to the fact that, in the lipopolysaccharide microheterogeneous complex of strain SKHM1-188, the number of LPS1 macromolecules is significantly greater (2 : 1) in relation to LPS2 than in strains Tb29 (0.53 : 1) and Ts22 (0.69 : 1). These data agree well with the results of polyacrylamide gel electrophoresis (Fig. 1b).

Summing up the results we obtained, it may be concluded that a characteristic feature of the LPS of the *S. meliloti* strains studied is the large content of glucose of nonglycan origin and KDO, as well as the presence of labile bonds in different sites of the LPS macromolecules, which is evidenced by the atypical pattern of their degradation with acetic acid.

The structure of the O-polysaccharide chain has been established for few rhizobial strains. For the LPS of *Rhizobium* (*R. leguminosarum* bv. *viciae,* bv. *trifolii, R. etli, R. tropici*), it was shown that the repeating O-chain unit consists of three to five different monosaccharides. As for the LPS of *Sinorhizobium (S. fredii, S. meliloti,* and *Sinorhizobium* sp. NGR234), Reuhs and colleagues suggested that the O-chain might be represented by the homopolymer glucan [1].

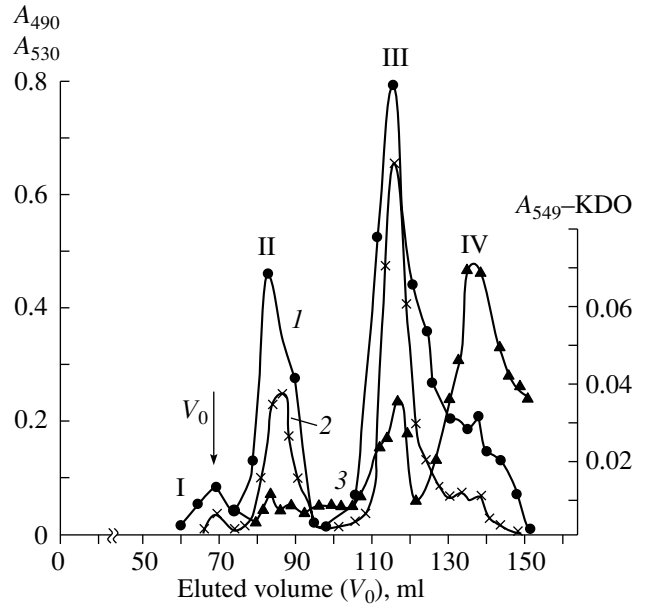

Fig. 3. Gel filtration on Sephadex G-25 of polysaccharides obtained as a result of partial degradation of *S. meliloti* Tb29 LPS with 1% acetic acid.

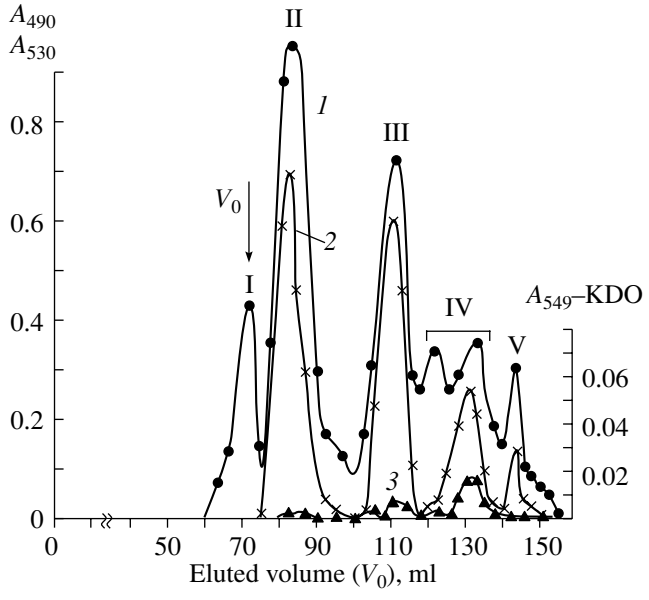

Fig. 4. Gel filtration on Sephadex G-25 of polysaccharides obtained as a result of partial degradation of *S. meliloti* Ts22 LPS with 1% acetic acid.

Table 4. Monosaccharide composition of the polysaccharide fractions (Sephadex G-25) of *S. meliloti* SKHM1-188 LPS partially degraded with 1% acetic acid

Monosaccharide	Fractions (MM, Da)				
	I (4000)	II (2700)	III (680)	IV (350)	V (180)
	% of the total peak area in GLC chromatograms				
Glucose	52.0	88.2	70.6	44.3	5.51
Galactose	16.5	4.4	16.5	30.3	12.2
Mannose	12.2	2.2	1.2	0.8	1.9
X_1 (T_{Glc} 0.53)	3.1	2.2	11.8	11.5	26.3
O_2 (O_{Glc} 0.47)	4.7	2.2	–	5.5	4.5
X_3 (T_{Glc} 0.43)	11.6	0.9	–	7.7	–
	μg/ml fraction				
Total carbohydrate content	20.2	134.0	94.0	53.0	98.0
Uronic acids	18.5	121.0	113.0	36.0	98.0
KDO	–	0.2	2.8	1.7	1.0

Note: Here and in Tables 5 and 6, a dash denotes absence of a component.

Table 5. Monosaccharide composition of the polysaccharide fractions (Sephadex G-25) of *S. meliloti* Tb29 LPS partially degraded with 1% acetic acid

Monosaccharide	Fractions (MM, Da)			
	I (>5000)	II (2500)	III (600)	IV (210)
	% of the total peak area in GLC chromatograms			
Glucose	43.3	82.8	71.8	36.5
Galactose	39.5	1.0	18.3	20.1
Mannose	3.9	2.9	5.6	6.5
X_1 (T_{Glc} 0.53)	4.5	8.1	4.2	28.5
X_2 (O_{Glc} 0.47)	8.9	3.5	–	5.1
X_3 (T_{Glc} 0.43)	–	1.7	–	3.3
	μg/ml fraction			
Total carbohydrate content	21.0	57.0	120.0	41.0
Uronic acids	3.0	67.0	142.0	19.0
KDO	0.3	0.6	1.5	2.5

The O-polysaccharide presence, size, and composition is of great importance in the recognition of rhizobia by the macrosymbiont [18, 19]. The specific LPS–host plant lectin interaction occurs when the bacteria have already penetrated into the root hair. Therefore, for them not to be recognized and agglutinated on the surface of the host cells prematurely, the specific sites of LPS binding to the lectin must be masked. Since it has not yet been established where precisely the specific sites are localized—on the LPS O-side chain or in its core region, it may be supposed that the O-polysaccharide, depending on its size, may perform, to a certain degree, a screening role, preventing the bacteria from agglutination on the roots at the early stages of the establishment of the between-symbiont interrelationships.

The reduced size of the O-PS, in addition to reducing its screening capacity, increases the relative lipid A content in the LPS macromolecule, increasing its hydrophobicity (water repellency). This results in an increase in the hydrophobicity of the cell surface of rhizobia, which is conducive to their nonspecific adhe-

Table 6. Monosaccharide composition of the polysaccharide fractions (Sephadex G-25) of *S. meliloti* Ts22 LPS partially degraded with 1% acetic acid

Monosaccharide	Fractions (MM, Da)				
	I (4600)	II (2900)	III (770)	IV (270)	V (180)
	% of the total peak area in GLC chromatograms				
Glucose	49.6	73.8	89.5	43.2	58.5
Galactose	10.3	11.3	4.8	44.8	18.1
Mannose	7.2	6.0	0.8	1.2	1.3
X_1 (T_{Glc} 0.53)	11.6	2.6	4.2	10.9	15.8
X_2 (O_{Glc} 0.47)	2.1	3.0	–	–	3.4
X_3 (T_{Glc} 0.43)	10.2	3.3			2.9
	μg/ml fraction				
Total carbohydrate content	72.0	162.0	112.0	62.5	56.0
Uronic acids	–	190.0	165.0	–	–
KDO	–	0.3	0.4	2.0	1.2

Table 7. Ratio of polysaccharide fractions of *S. meliloti* LPS partially degraded with 1% acetic acid

Strains	Fractions	PS1/PS2 ratio*
SKHM1-188	PS1 (fraction I, 4000 Da + fraction II, 2700 Da), PS2 (fraction III, 680 Da)	2 : 1
Tb29	PS1 (fraction II, 2500 Da), PS2 (fraction III, 600 Da)	0.53 : 1
Ts22	PS1 (fraction II, 2900 Da), PS2 (fraction III, 770 Da)	0.69 : 1

* Calculated by the fraction areas of the elution profiles presented in Figs. 2–4.

sion on the roots of a host plant. As a result, the rate of penetration of the bacteria into the root hair is decreased, due to which other competitive homologous rhizobial strains infect the plant more successfully.

Thus, a decrease in the nodulation competitiveness in the LPS mutants studied when they were inoculated jointly with the test (SKHM1-48) [4] or parent (SKHM1-188) [5] *S. meliloti* strains may be determined by the loss of the ability to synthesize the full set of the LPS O-polysaccharide forms, which might perform different biological functions.

The biological properties of LPS also depend on the qualitative composition of fatty acids and their content in lipid A [20]. Therefore, changes in the fatty acid ratios in the LPS of the mutants, compared to the parent strain, influence the infectivity of rhizobia and their nodulation competitiveness.

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