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Characterization of the Lipopolysaccharide from a *Rhizobium phaseoli* Mutant That Is Defective in Infection Thread Development

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The lipopolysaccharide (LPS) from a *Rhizobium phaseoli* mutant, CE109, was isolated and compared with that of its wild-type parent, CE3. A previous report has shown that the mutant is defective in infection thread development, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis shows that it has an altered LPS (K. D. Noel, K. A. VandenBosch, and B. Kulpaca, J. Bacteriol. 168:1392–1462, 1986). Mild acid hydrolysis of the CE3 LPS released a polysaccharide and an oligosaccharide, PS1 and PS2, respectively. Mild acid hydrolysis of CE109 LPS released only an oligosaccharide. Chemical and immunochemical analyses showed that CE3-PS1 is the antigenic O chain of this strain and that CE109 LPS does not contain any of the major sugar components of CE3-PS1. CE109 oligosaccharide was identical in composition to CE3-PS2. The lipid A's from both strains were very similar in composition, with only minor quantitative variations. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of CE3 and CE109 LPSs showed that CE3 LPS separated into two bands, LPS I and LPS II, while CE109 had two bands which migrated to positions similar to that of LPS II. Immunoblotting with anti-CE3 antiserum showed that LPS I contains the antigenic O chain of CE3, PS1. Anti-CE109 antiserum interacted strongly with both CE109 LPS bands and CE3 LPS II and interacted weakly with CE3 LPS I. Mild-acid hydrolysis of CE3 LPS I, extracted from the polyacrylamide gel, showed that it contained both PS1 and PS2. The results in this report showed that CE109 LPS consists of only the lipid A core and is missing the antigenic O chain.

Bacteria of the genus *Rhizobium* are able to form nitrogen-fixing symbiotic relationships with legume plants. As gram-negative bacteria, they have the usual surface polysaccharides consisting of extracellular polysaccharides, capsular polysaccharides, and lipopolysaccharides (LPSs). All of these molecules have been hypothesized to play a role in the symbiotic infection process (for a review, see reference 6).

The LPSs have been the least studied of all the above-mentioned *Rhizobium* polysaccharides. Composition studies show that *Rhizobium* LPSs vary greatly among different species as well as among strains of a single species (6, 9, 33). Lipopolysaccharides from *R. phaseoli*, *R. leguminosarum*, and *R. trifolii* have a characteristic structural motif revealed by mild-acid hydrolysis. A polysaccharide (PS1) and an oligosaccharide (OS; PS2) are released from lipid A (7). The PS1 polysaccharide has been termed the O antigen (7) and varies in composition from strain to strain. The PS2 OSs, termed the LPS core, exhibit conserved composition. All PS2s migrate similarly on gel filtration and are composed largely of galacturonic acid with smaller amounts of mannose, galactose, and glucose (7, 10) and 2-keto-3-deoxy-octonic acid (KDO; 10). Recently, the structure of the major core component from an *R. trifolii* LPS has been determined (R. W. Carlson, R. Hollingsworth, and F. B. Dazzo, submitted for publication). It consists of two terminal galacturonic acid residues α-linked to positions 4 and 7 of KDO (Carlson et al., submitted). A minor core component of this LPS contains mannose, galactose, KDO, and galacturonic acid (Carlson et al., submitted). In most cases, the rhizobial O antigens are likely to be complex OSs rather than a polysaccharide formed by the polymerization of a repeating OS (7, 10). In addition, these O antigens commonly contain methylated sugars and methylated amino sugars, and when heptose is present, it is found in the O antigen and not in the core OS (7, 10). For several *R. trifolii* LPSs, both the O antigen and core have KDO at their reducing ends, indicating that both are linked to the remainder of the LPS molecule via a KDO residue (10; Carlson et al., submitted). These results are unlike those for *Salmonella* and *Escherichia coli* LPSs, which consist of a heptose-containing core OS linked via KDO to the lipid A and a repeating OS O antigen which is linked to the core OS (32).

Investigations of the role of LPSs in symbiosis have been based largely on the ideas that a rhizobial symbiont attaches to the host root in a host-specific manner and that this specific attachment is mediated via the host lectin. Some of these reports present data showing that lectin from the host appears to bind LPS only from the symbiont bacteria (20, 21). It has also been reported that LPS can inhibit binding of the bacterial symbiont to the host root (22). Whether or not these data support a role for LPS in symbiosis is still uncertain, since the data concerning the specificity of attachment and binding of host lectin to rhizobial symbiont are ambiguous. Some reports suggest that there is no specificity in attachment (5, 25). Other reports suggest that there is nonspecific attachment with high levels of bacteria and specific attachment with low levels of bacteria (3, 13).

In addition to the above-mentioned studies, changes have been reported in the composition of LPSs from *R. trifolii* Nod$^-$ mutants which lack the symbiotic (Sym) plasmid or

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have a deletion in this plasmid (10, 11, 27). However, since the Sym plasmid probably carries many genes in addition to those required for nodulation, it is difficult to say whether or not these data imply a role for LPSs in symbiosis. Thus, while both the binding and composition studies are interesting, they do not prove that a role for LPS in symbiosis exists, nor do they identify the nature of that role.

A recent report describes two mutants of *R. phaseoli* which form nodules with a characteristic aberration in development (24). These nodules contain infection threads and bacteria; however, the infection threads develop abnormally and abort (24). The mutants have been shown to have an altered LPS. Furthermore, the defect in LPS and the defect in symbiosis are due to a single mutation in each mutant (24). When the mutant LPSs are analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE), they lack a higher-molecular-weight band, LPS I, (previously called SacI [24]), but contain a lower-molecular-weight band (LPS II) which is present in both the parent and the mutant LPSs (24). For other *Rhizobium* spp., LPS I is the complete form of the LPS which carries the O-antigen, while LPS II is LPS which lacks the O antigen (7, 8, 10). Thus, it is likely that these *R. phaseoli* LPS mutants lack the O antigen portion of their LPSs. In this report we describe the further chemical and immunochemical characterization of normal LPS and LPS from one of these symbiotically defective mutants.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Bacterial strains used were *R. phaseoli* CE3, the Str^r parent strain, and mutant CE109. The mutant was obtained by Tn*5* mutagenesis as previously described (24). The bacteria were grown at 30°C in 0.5% tryptone, 0.3% yeast extract, and 10 mM $CaCl_2$ (TY).

Purification of LPSs. Bacteria were grown at 30°C in 10- to 50-liter batches of TY broth, aerated by shaking at 240 rpm in Erlenmeyer flasks filled to one-half capacity, harvested at early stationary phase with a Sharples centrifuge, washed three times in 100 to 500 ml of 1% NaCl, and washed twice in 100 to 500 ml of distilled water. At the time of harvest, dilutions of the cultures were plated on agar media to identify contaminants. From CE3 cultures, all colonies on TY were Str^r and required calcium to grow in tryptone media, a characteristic requirement of these rhizobia. The test for strain CE109 was similar, except that all colonies on TY were Str^r Km^r. The washed cell pellet was extracted with hot phenol-water as previously described (31). The material extracted into the water layer was treated with RNase A and was further purified by column chromatography in EDTA-triethylamine as previously described (9). Fractions in the KDO-containing peak were combined, dialyzed against deionized water for 5 to 7 days, and freeze-dried. The yields of LPS were 1.4 and 2.5 mg/10^{12} cells for CE109 and CE3 cultures, respectively. The polysaccharide portions of the LPSs were obtained by mild-acid hydrolysis with 2% acetic acid at 100°C (7, 28). The precipitated lipid A was centrifuged, and the water layer was extracted with chloroform. The material extracted into the water layer was freeze-dried and applied to a Sephadex G-50 column (7, 28). Fractions from the G-50 column were combined in two portions, reflecting the hexose peaks.

In some cases, the LPS from column chromatography was purified further by SDS-PAGE before mild-acid hydrolysis. LPS I was separated from LPS II by PAGE. After electrophoresis, a small strip of the gel slab was silver stained to locate LPS I. The LPS I was cut out of the gel and passed through a 12-cm^3 syringe with no needle attached. The fragments were frozen in liquid nitrogen and pulverized further by mortar and pestle. Ten volumes of 50 mM Tris hydrochloride–2% SDS (pH 6.8) buffer was added, and the suspension was stirred at 100°C for 10 min. After incubation at room temperature overnight, it was again heated at 100°C for 10 min, and centrifuged at 27,000 × *g* for 10 min at 15 to 25°C. The supernatant was filtered through a 0.2-μm-pore-size membrane filter (Millipore Corp.) and dialyzed against water for 1 week. The dialysate was lyophilized. The resulting powder was extracted four times with absolute ethanol to remove SDS. The final pellet was dried by vacuum and assayed for SDS (2) and KDO content.

Composition analysis. The sugar compositions were determined by acetylation analysis. This procedure consisted of acid hydrolysis of the polysaccharides, preparation of their alditol acetates, and gas chromatography (GC; 1) with a SP2330 15-m capillary column (Supelco, Bellefonte, Pa.). Identification and quantitation were performed by comparing results to authentic standards. Inositol was added to all samples as an internal standard. Identification of 2-*O*-methyl-6-deoxyhexose, 3-*O*-methyl-6-deoxyhexose, and 2-amino-2,6-dideoxyhexose was accomplished with combined GC-mass spectrometry (MS). Uronic acids were identified by reducing the carboxyl groups prior to acetylation analysis. Increases in the quantity of or the appearance of a particular hexose compared with results from the non-carboxyl-reduced sample show that the hexose is a hexuronic acid in the original polysaccharide. Hexuronic acid was reduced by treating the sample in methanolic HCl, followed by reduction with sodium borohydride (14). Uronic acids were quantitated by the method of Blumenkrantz and Asboe-Hansen (4), with glucuronic acid as a standard. Acyl groups, pyruvate, and KDO were assayed by colorimetric assays (18, 23, 30), with glucose pentaacetate, pyruvate, and KDO, respectively, as standards.

Fatty acids were identified and quantitated by slightly modifying a previously described procedure (26). Lipid A samples, approximately 500 μg each, were hydrolyzed in 4 M HCl for 2 h at 100°C, hydrolysed in 4 M NaOH for 2 h at 100°C, acidified with HCl, and extracted three times with petroleum ether. Before hydrolysis, 100 μg of lauric acid was added to each sample as an internal standard. The ether layers were evaporated to dryness with filtered air at 4°C. Methyl esters of the fatty acids were prepared by adding 500 μl of BF_3-methanol, heating at 100°C for 2 min, cooling on ice, and extracting with petroleum ether. The samples were evaporated with filtered air at 4°C, dissolved in dichloromethane, and analyzed by GC. The GC was done on an SPB1 30-m capillary column (Supelco) with a temperature program of 150 to 250°C at 4°C/min. The fatty acids were identified by comparing retention times to that of a standard fatty acid mix obtained from Supelco, as well as by GC-MS analysis.

PAGE. Discontinuous slab gel electrophoresis was performed by the method of Hitchcock and Brown (19). The gels were stained by the silver staining procedure previously described (19).

Preparation of antisera. Rabbits were injected with 0.2 ml of 1.0 mg of purified LPS or 2 × 10^9 bacteria per ml, washed twice, and suspended in phosphate-saline buffer. Injections were given weekly for 3 weeks. Two days after the last injection, the rabbits were bled. The blood was allowed to clot at 25°C for 1 h and at 4°C overnight. The serum below

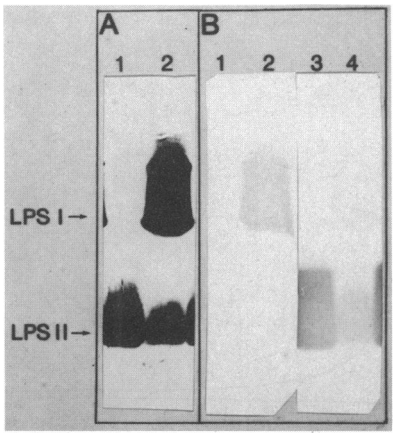

FIG. 1. Purified LPS from strains CE3 (wild type) and CE109 (mutant) subjected to SDS-PAGE. (A) Silver staining. Lane 1, 40 μg of CE109 LPS; lane 2, 70 μg of CE3 LPS. (B) Immunoblot reacted with antiserum against strain CE3 (lanes 1 and 2) or against strain CE109 (lanes 3 and 4). Lanes 1 and 3, 40 μg of CE109 LPS; lanes 2 and 4, 70 μg of CE3 LPS. The positions of LPS I and LPS II are indicated by arrows.

TABLE 1. Compositions of LPSs from *R. phaseoli* CE3 and CE109

Component	% of LPS mass from strain	
	CE3	CE109
2-*O*-Methyl-6-deoxyhexose	2.5 ± 0.03	ND[a]
3-*O*-Methyl-6-deoxyhexose	4.8 ± 0.07	ND
Fucose	6.3 ± 0.3	ND
Mannose	4.0 ± 0.1	3.5 ± 0.2
Galactose	2.3 ± 0.1	2.6 ± 0.2
Glucose	0.8 ± 0.1	1.8 ± 0.7
2-Amino-2,6-dideoxyhexose	1.5 ± 0.2	ND
Glucuronic acid		ND
Galacturonic acid		12 ± 0.0
Glucuronic and galacturonic acid[b]	23 ± 0.4	
2-Keto-3-deoxyoctonate	5.2 ± 0.03	4.1 ± 0.05
Pyruvate	ND	1.5
Acyl groups	3.6	2.9
Lipid A[c]	42	57

[a] ND, Not detected.
[b] Uronic acid in LPS from strain CE3 consists of both glucuronic and galacturonic acid.
[c] Lipid A was weighed after it was released from the LPS by mild-acid hydrolysis.

the clot was centrifuged at 3,500 × *g* for 10 min. The supernatant solution was heated at 55°C for 30 min and stored at 4°C.

Immunoblot procedure. The LPSs were transferred electrophoretically from a slab gel to nitrocellulose paper with a Tris-glycine-methanol solution. The nitrocellulose paper was soaked in the appropriate antisera and then stained with peroxidase-conjugated protein A (Fig. 1) or goat anti-rabbit antibodies (Fig. 2; see also Fig. 4). The details of this procedure were as previously described (10, 12).

ELISA procedure. Enzyme-linked immunosorbent assays (ELISA) were performed as previously described (10, 15, 29). Polystyrene microtiter plates were coated with LPS. Dilutions of polysaccharide PS1 were incubated in 1,000-fold-diluted antiserum at 37°C overnight, and this mixture was added to the microtiter plates. After incubation for 1.5 h at room temperature, the plates were washed and alkaline phosphatase-conjugated anti-rabbit goat antisera was added at a final concentration of 10 μg/ml. After incubation for 3 h at 37°C, the plates were washed and enzyme activity was detected by adding *p*-nitrophenyl phosphate substrate.

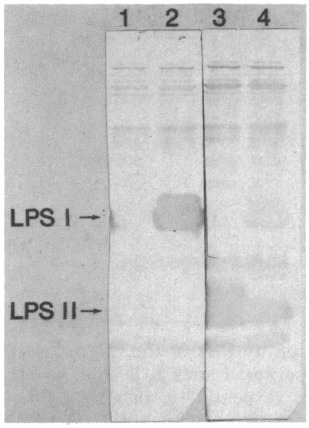

FIG. 2. Washed CE3 (wild type) and CE109 (mutant) broth cultures extracted into SDS buffer and separated by SDS-PAGE. The immunoblot was reacted with anti-CE3 (lanes 1 and 2) or anti-CE109 (lanes 3 and 4) antiserum. Lanes 1 and 3, CE109 cells; lanes 2 and 4, CE3 cells.

RESULTS

Composition analysis. LPS compositions are given in Table 1. LPS from the parent strain, CE3, contained several sugars not found in the mutant LPS. These sugars were 2-*O*-methyl-6-deoxyhexose, 3-*O*-methyl-6-deoxyhexose, fucose, glucuronic acid, and 2-amino-2,6-dideoxyhexose. Mannose, galactose, glucose, galacturonic acid, KDO, and acyl groups were found in both LPSs. It is possible that the small amount of glucose was due to slight contamination by a glucan, since the polysaccharides released from the LPSs by mild-acid hydrolysis did not contain significant levels of glucose (see below). Pyruvate was detected in small amounts in the mutant LPS but not in the parent LPS. Identification of the uronic acids by reduction-acetylation resulted in a three- to fourfold increase in the amounts of both galactose and glucose found in the parent LPS and a two- to threefold increase in the amount only of galactose found in the mutant LPS. Thus, the parent LPS contained both glucuronic and galacturonic acids, while the mutant LPS contained only galacturonic acid.

The lipid A's were isolated by mild-acid hydrolysis of the LPSs. Each lipid A precipitated during this process and was isolated by centrifugation and extraction with chloroform. The fatty acid compositions of the lipid A's from each LPS were very similar to one another and are given in Table 2. There were three major components: β-hydroxymyristic acid, β-hydroxypalmitic acid, and β-hydroxystearic acid. Each lipid A had three minor unidentified components (designated a, b, and c). One of these was another β-hydroxy fatty acid, since GC-MS of this component gives a fragment which is characteristic of these molecules (*m/z*, 103) (16). There were small quantitative differences between these two lipid A's. The CE109 lipid A contained lower amounts of β-hydroxystearic acid and stearic acid than did the CE3 lipid A.

Mild-acid hydrolysis of CE3 LPS produced a higher-molecular-weight polysaccharide and a lower-molecular-

TABLE 2. Composition of lipid A from LPSs of *R. phaseoli*[a]

Component	% of lipid A from strain	
	CE3	CE109
3-OH C_{14}	48	54
3-OH C_{16}[b]	14	10
3-OH C_{18}[b]	14	14
$C_{18:1}$	6.1	5.4
C_{18}	3.7	1.7
3-OH C_{15}[b]	1.7	1.7
C_{16}	2.0	1.9
a	3.2	3.4
b	3.9	4.4
c	1.6	1.5
Glucosamine[c]	6.0	6.3

[a] Fatty acid components are relative percentages calculated from the areas of the GC peaks. If all the fatty acid components have the same response factors, then the components listed above account for 43 and 45% of the CE3 and CE109 lipid A masses, respectively.

[b] Chain lengths of these 3-hydroxy fatty acids were determined by the linear relationship between the logarithm of the retention time and the number of carbons in the fatty acid when hydrolysed at constant temperature.

[c] Glucosamine is given as percentage of the lipid A mass.

weight OS (PS1 and PS2, respectively), which were separated by gel filtration chromatography on Sephadex G-50 (24). Hydrolysis of CE109 LPS produced only an OS (CE109 OS) which eluted at the same volume as CE3 PS2 (24). Each fraction was purified further by gel filtration chromatography with Sephadex G-25. Table 3 shows the compositions of the polysaccharides released from the LPSs by mild-acid hydrolysis. The PS1 fraction was found only in LPS from CE3. Its main sugars were 2-*O*-methyl-6-deoxyhexose, 3-*O*-methyl-6-deoxyhexose, fucose, and glucuronic acid, with lower amounts of mannose, KDO, and 2-amino-2,6-dideoxyhexose. The CE3 PS2 and CE109 OS fractions both contained galacturonic acid as the major component, with lower amounts of mannose, galactose, and KDO. It should also be noted that monomeric KDO was produced during mild-acid hydrolysis and could be partially separated from CE3 PS2 and CE109 OS by gel filtration chromatography with Sephadex G-25. We found that prereducing CE3 PS1, CE3 PS2, and CE109 OS fractions reduced the amount of KDO in these samples to less than 0.5% of the normal amount, indicating that the KDO is at the reducing end of each of these OSs.

Immunological studies. Antisera were raised against washed cell cultures of strains CE3 and CE109. LPSs were

TABLE 3. Composition of polysaccharides released by mild-acid hydrolysis of *R. phaseoli* LPSs[a]

Component	% in polysaccharides from strain:		
	CE3 PS1	CE3 PS2	CE109 OS
2-*O*-Methyl-6-deoxyhexose	12	ND	ND
3-*O*-Methyl-6-deoxyhexose	23	ND	ND
Fucose	22	ND	ND
Mannose	6.1	19	19
Galactose	ND	18	19
Glucose	Tr	Tr	Tr
2-Amino-2,6-dideoxyhexose	9.2	ND	ND
Glucuronic acid	22	ND	ND
Galacturonic acid	ND	43	43
2-Keto-3-deoxyoctonate	4.5	19	19

[a] Compositions are relative percentages. The components listed above account for 86, 62, and 52% of the masses of CE3 PS1, CE3 PS2, and CE109 OS fractions, respectively. ND: Not detected; Tr, trace.

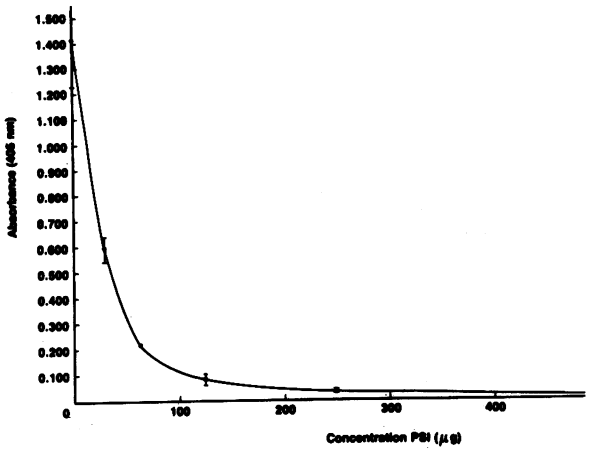

FIG. 3. ELISA showing inhibition by PS1 of the interaction between CE3 LPS and anti-CE3 antiserum.

prepared from these strains and subjected to SDS-PAGE. Antisera prepared against wild-type CE3 reacted strongly with LPS I (the slower-migrating wild-type band) and very weakly against LPS II (Fig. 1). This weak interaction with LPS II is visible on the original immunoblot but not in the photograph shown in Fig. 1. Antisera against mutant strain CE109 reacted strongly against LPS II and weakly against LPS I (Fig. 1B, lanes 3 and 4).

To compare the LPS antigens with other antigens of these *R. phaseoli* strains, washed cells were extracted in SDS and separated by PAGE (Fig. 2). The reaction of antisera against CE3 revealed an intense band that comigrates with LPS I. This band was missing in CE109. It appears that LPS I carries the dominant antigen(s) of the wild-type cell. An inhibition ELISA showed that PS1 from strain CE3 strongly inhibited the interaction between CE3 LPS and anti-CE3 antiserum (Fig. 3). Figure 4 shows inhibition by PS1 of the interaction between LPS I and anti-CE3 on an immunoblot.

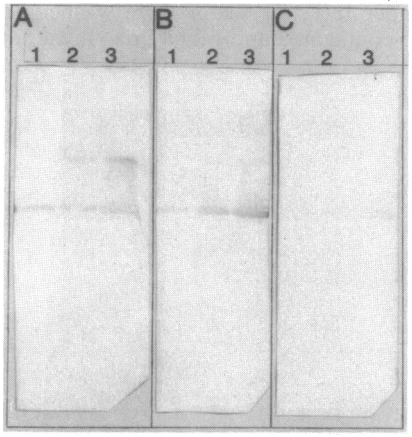

FIG. 4. Inhibition by PS1 of the interaction between CE3 LPS and anti-CE3 antiserum. Lanes 1, 2, and 3 were loaded with 0.39, 1.56, and 6.25 μg, respectively, of CE3 LPS. After SDS-PAGE, immunoblots were reacted with anti-CE3 (A), anti-CE3 preincubated with PS2 (B), or anti-CE3 preincubated with PS1 (C). In the preincubations, antiserum at a dilution of 1/1,000 was incubated overnight at 37°C with 1.0 mg of PS2 or PS1 per ml or with no addition. The final concentration of antiserum (a 10^5-fold dilution) reacted with the blots was 300-fold less than for Fig. 1 and 2.

Thus, PS1 was the immunodominant antigen of CE3 and was missing in the mutant CE109. The presence of CE3 PS1 in LPS I was verified by extracting and characterizing this material from the polyacrylamide gel. Both PS1 and PS2 were isolated from LPS I by mild-acid hydrolysis and gel filtration chromatography.

Strain CE109 lacks LPS I. However, when smaller amounts of SDS extract or the phenol-water extract from CE109 were run on SDS-PAGE gels, we found another band just above the LPS II band. This band was visible when stained with silver and with antisera to CE109. A band at the same position was detected occasionally from wild-type LPS preparations, but the band from the mutant is more prominent.

DISCUSSION

A previous report showed that LPS from the wild-type CE3 strain consists of two forms of LPS, LPS I and LPS II, while the LPS of the mutant CE109 consists of only LPS II (24). The data in this report showed that the component missing in mutant LPS is the antigenic O chain, PS1. These data can be summarized as follows. (i) Immunoblots showed that anti-CE3 antiserum bound to LPS I but bound very weakly to LPS II of CE3 and to CE109 LPS. (ii) The inhibition ELISA showed that PS1 polysaccharide, released by mild-acid hydrolysis of CE3 LPS, strongly inhibited the binding of anti-CE3 antisera to CE3 LPS. (iii) Composition analysis of LPSs from both CE3 and CE109, as well as analysis of the isolated OSs released by mild-acid hydrolysis, showed that mutant LPS does not contain PS1 or any of the major glycosyl residues found in PS1. In addition, the data in this report showed that the polysaccharide portion of mutant LPS, CE109 OS, and CE3 PS2 have identical compositions and gel filtration elution profiles. Furthermore, anti-CE109 antisera preferentially bound to LPS II from CE3. Hence, both composition and immunology suggest that CE3 PS2 and CE109 OS are very similar in structure. Experiments are in progress to determine the exact structure of these OSs.

In a related rhizobial strain, 128C53, which nodulates pea roots, LPS I was separated from LPS II by SDS-PAGE and shown to contain the sugars of both PS1 and PS2 for that strain (7). The fact that loss of the O antigen from mutant CE109 leads to an LPS whose sugar composition matches that of CE3 PS2 is consistent with this result. In addition, we have shown that both PS1 and PS2 can be isolated from CE3 LPS I. However, antiserum against CE109 cells reacts weakly with CE3 LPS I, whereas the reaction with CE3 LPS II is intense (Fig. 1). At least three possibilities could explain this result. One is that perhaps there is more PS2 in the LPS II band than in LPS I; either LPS II is more abundant than LPS I, or not all LPS I molecules contain PS2. Another possibility is that LPS I molecules carry incomplete or modified PS2. This possibility might explain the two "LPS II" bands of CE109. Finally, PS2 might be more accessible in LPS II than in LPS I.

To date, eight other mutants isolated from *R. phaseoli* CE3 have been found to be defective in nodule development and LPS (P. Pachori, J. R. Cava, and K. D. Noel, unpublished data). All lack LPS I except one, which has substantially decreased LPS I content. Tentatively, it appears that the ability to synthesize LPS molecules containing the O antigen is essential for carrying infection of beans beyond an early stage. On the other hand, normal LPS appears to be unnecessary for earlier events in bean nodulation, such as attachment to the root surface, root hair curling, and induction of root cortical-cell division to produce a nodule structure (24). The role of intact LPS in infection has yet to be determined. One possibility is that the O antigen, or a nodule-specific modification of it, is recognized by the plant as a positive signal to sustain infection. Another possibility is that LPS acts as a barrier against host toxins or otherwise suppresses host defense mechanisms. This last possibility is consistent with the avirulence of LPS mutants of the plant pathogen *Pseudomonas solanacearum* (17). It may be that successful gram-negative interactions with plants involve general constraints on LPS structure.

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