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Heterogeneity of *Rhizobium* Lipopolysaccharides

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The lipopolysaccharides (LPSs) from strains of *Rhizobium leguminosarum*, *Rhizobium trifolii*, and *Rhizobium phaseoli* were isolated and partially characterized by mild acid hydrolysis and by polyacrylamide gel electrophoresis. Mild acid hydrolysis results in a precipitate which can be removed by centrifugation or extraction with chloroform. The supernatant contains polysaccharides which, in general, are separated into two fractions (LPS1 and LPS2) by Sephadex G-50 gel filtration chromatography. The higher-molecular-weight LPS1 fractions among the various *Rhizobium* strains are highly variable in composition and reflect the variability reported in the intact LPSs (R. W. Carlson and R. Lee, Plant Physiol. **71**:223–228, 1983; Carlson et al., Plant Physiol. **62**:912–917, 1978; Zevenhuizen et al., Arch. Microbiol. **125**:1–8, 1980). The LPS1 fraction of *R. leguminosarum* 128C53 has a higher molecular weight than all other LPS1 fractions examined. All LPS2 fractions examined are oligosaccharides with a molecular weight of ca. 600. The major sugar component of all LPS2 oligosaccharides is uronic acid. The LPS2 compositions are similar for strains of *R. leguminosarum* and *R. trifolii*, but the LPS2 from *R. phaseoli* was different in that it contained glucose, a sugar not found in the other LPS2 fractions or found only in trace amounts. Polyacrylamide gel electrophoretic analysis shows that each LPS contains two banding regions, a higher-molecular-weight heterogeneous region often containing many bands and a lower-molecular-weight band. The lower-molecular-weight bands of all LPSs have the same electrophoretic mobility, which is greater than that of lysozyme. The banding pattern of the heterogeneous regions varies among the different *Rhizobium* strains. In the case of *R. leguminosarum* 128C53 LPS, the heterogeneous region is of a higher molecular weight than is this region from all other *Rhizobium* strains examined and consists of many bands separated from one another by a small and apparently constant molecular weight interval. When the heterogeneous region of *R. leguminosarum* 128C53 LPS was cut from the gel and analyzed, its composition was found to be that of the intact LPS, whereas the lower-molecular-weight band contains only sugars found in the LPS2 oligosaccharide. In the case of *R. leguminosarum* 128C63 and *R. trifolii* 0403 LPSs, the heterogeneous regions are similar and consist of several bands separated by a large-molecular-weight interval with the major band of these heterogeneous regions having the lowest molecular weight with an electrophoretic mobility near that of beta-lactoglobulin. The hetergeneous region from *R. phaseoli* 127K14 consists of several bands with electrophoretic mobilities near that of beta-lactoglobulin, whereas this region from *R. trifolii* 162S7 shows a continuous staining region, indicating a great deal of heterogeneity. The results described in this paper are discussed with regard to the reported properties of *Escherichia coli* and *Salmonella* LPSs.

Rhizobia are gram-negative bacteria which form a nitrogen-fixing symbiotic relationship with legume plants. As gram-negative bacteria they have the usual surface polysaccharides consisting of extracellular polysaccharides, capsular polysaccharides (CPSs), and lipopolysaccharides (LPSs). All of these molecules have been thought to play a role in the specific attachment of the symbiont bacteria to the legume host (for review, see references 2, 4, and 7). Furthermore, it has been hypothesized that this attachment to the host legume root occurs via the host plant lectin (see the above references for review).

In addition to many reports which implicate a role for the extracellular polysaccharides or CPSs in this attachment process, there are several reports which suggest that the LPSs are important in the symbiotic process. Pea lectin has been reported to bind to the symbiont *Rhizobium leguminosarum* LPS but not to the LPSs from nonsymbiont *Rhizobium* species (13, 14). Similar results were obtained for the LPS from *Rhizobium meliloti* and for the lectin from *Medicago sativa* (13). It has also been reported that LPS and CPS from *R. leguminosarum* inhibit the binding of these bacteria to the host root (15). More recently it has been reported that the LPS from *Rhizobium trifolii* serves as a receptor for trifoliin, the clover lectin (11). Data also have been presented which suggest that two forms of LPS are present, one of which serves as the major receptor for trifoliin and appears during the late exponential phase of growth (11).

Differences in the composition between the LPSs from nodulating and nonnodulating mutants of *R. trifolii* have been reported (18, 19). This change in LPS composition has been correlated with the elimination of a plasmid from the nodulating strain (18). The major difference between these two LPSs is that the LPS from the nonnodulating mutant contains fucose as a major sugar and lacks glucose, whereas the parent LPS does not contain fucose but contains glucose as a major sugar. Since the missing plasmid in the mutant probably contains many genes, in addition to the nodulation genes, it is difficult to say whether these data imply a role for LPSs in symbiosis.

Recently, Carlson and Lee (5) compared the surface polysaccharides from a nonnodulating mutant of *R. leguminosarum* with the polysaccharides from the parental strain. The mutant is unable to produce any of the parental-type extracellular polysaccharides or CPSs. However, the LPS from the mutant is identical in composition to the parent LPS (5). Additional data also suggest that the major polysaccharides released from the mutant and parent LPSs by mild acid hydrolysis, possibly the O-antigen, have identical structures (R. M. Carlson, manuscript in preparation). Since the mutant is defective in that it does not appear to attach to the

host pea root hairs (C. Napoli, personal communication), the suggestion is that this polysaccharide component of the LPS does not play a role in attachment. The data do not rule out the possibility that LPSs play a role in later symbiotic steps. In fact, there are reports in the literature which suggest that the LPSs isolated from bacteroids may have a different composition than the LPSs isolated from cultured bacteria (17, 22).

Data have suggested that there are multiple forms of *Rhizobium* LPSs (4). If multiple forms of LPSs exist, then it is possible that one form is important for symbiosis, whereas the other form is not important, as suggested by Hrabak et al. (11). In this report we show that *Rhizobium* LPSs are highly heterogeneous molecules for strains of *R. leguminosarum*, *R. trifolii*, and *Rhizobium phaseoli*. The heterogeneity of the LPSs may be due to one or a combination of the following factors: different aggregation states, variable numbers of O-antigen repeating units, and multiple types of LPS molecules.

MATERIALS AND METHODS

Bacterial strains. All bacterial strains were obtained from Peter Albersheim, University of Colorado. The original source of the *R. leguminosarum* and *R. phaseoli* strains was Joe Burton, Nitragin Co., Milwaukee, Wis. *R. trifolii* 0403 and 162S7 were originally obtained from Carolyn Napoli.

Growth conditions. Bacteria were grown to the early stationary phase as previously described (5, 6).

LPS isolation. LPSs were isolated by the phenol-water extraction procedure (25) which was modified as previously described (5, 6).

Mild acid hydrolysis. The polysaccharide portion of the LPSs was separated from the lipid-A by mild acid hydrolysis in 1% acetic acid at 100°C for 1 h (20). For each LPS, the lipid-A formed a precipitate which was removed by centrifugation. The supernatant, which contains the polysaccharide, was extracted with chloroform to remove any remaining lipid-A, and the aqueous phase was freeze-dried before gel filtration chromatography.

Composition analyses. The hexose compositions were determined by acid hydrolysis of the polysaccharides, reduction of the monosaccharides produced by hydrolysis, and preparation of the alditol acetate derivatives, followed by gas chromatographic analysis (1). Uronic acid was determined by the method of Blumenkrantz and Asboe-Hansen (3). 2-Keto-3-deoxyoctonic acid (KDO) was assayed by the method of Weissbach and Hurwitz (24).

Gel electrophoresis. Discontinuous slab gel electrophoresis, using sodium dodecyl sulfate, was performed by the method of Hitchcock and Brown (10). The gels were stained for LPSs by the silver-staining technique described by Hitchcock and Brown (10). Any other conditions are described in the figure legends.

Composition analysis of the LPSs separated by gel electrophoresis. LPS (100 μg) was placed into each of five wells, and electrophoresis was carried out as described above. One well was sliced away from the gel and stained for carbohydrate by the periodic acid-Schiff base method as described by Fairbanks et al. (8). The remainder of the gel was placed in a 25:65:10 solution of isopropanol-water-acetic acid overnight. The gel was then placed in deionized water, and the water was changed each hour for ca. 3 h. Gel regions corresponding to the heterogeneous region and the lower band (see Fig. 3) were cut from two wells, sliced into small pieces, and placed in test tubes. Inositol (20 μg) was added to each tube as an internal standard. One milliliter of 2 N trifluoroacetic acid was added to each tube. The tubes were sealed and heated at 121°C for 2 h. The trifluoroacetic solutions were transferred to clean tubes and blown dry with filtered air at 45°C. Methanol (1 ml) was added to each tube and then blown dry with filtered air. The residue in each tube was then reduced with sodium borohydride, acetylated, and analyzed by gas chromatography as previously described (1). The upper and lower banding regions were cut from the remaining two wells and placed in test tubes as described above. One milliliter of 1% acetic acid was placed in each tube, and the sealed tubes were heated at 100°C for 1 h. The acetic acid solutions were transferred to new test tubes, blown dry with filtered air, and assayed for KDO and uronic acid as described above.

Gel filtration chromatography. Sephadex G-50 and G-25 gel filtration columns were run, using deionized water as the solvent. All other conditions are described in the figure legends.

RESULTS

Effect of mild acid hydrolysis on *Rhizobium* LPSs. Mild acid hydrolysis of all fast-growing *Rhizobium* LPSs examined results in a precipitate which can be removed by centrifugation or extraction with chloroform. The aqueous supernatant contains polysaccharides. When applied to a Sephadex G-50 gel filtration column, this material results in the elution profiles shown in Fig. 1. In each case, polysaccharides are released which elute from the G-50 column between the void volume and the bed volume. When intact LPS is applied to the G-50 column, it elutes at the void volume. The polysaccharides released from *R. leguminosarum* 128C53 LPS elute as two peaks, LPS1 and LPS2 (Fig. 1). The LPS1 from this

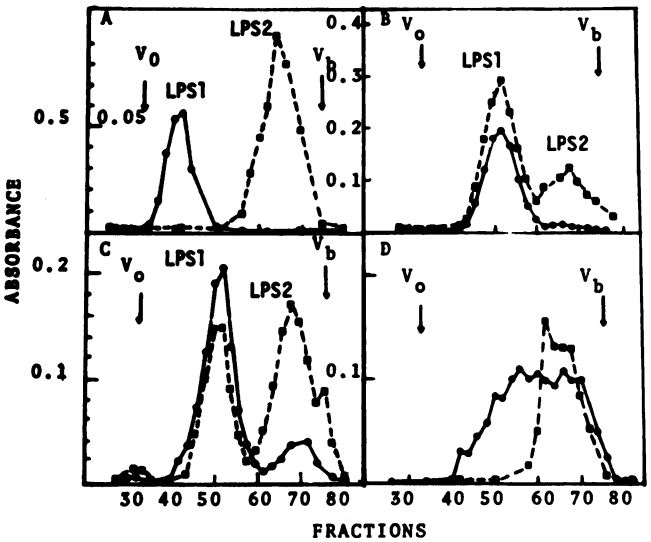

FIG. 1. Sephadex G-50 gel filtration elution profile of the polysaccharides released from the LPSs by mild acid hydrolysis. All polysaccharides were run on the same column (diameter, 1.3 cm; height, 60 cm). Each sample was applied in a total volume of 0.5 ml. The flow rate was ca. 0.2 ml/min. The void volume (V_O) and bed volume (V_b) are as indicated. The solid line represents hexose absorbance at 620 nm as measured by the anthrone assay, and the dashed line represents the uronic acid absorbance at 520 nm as measured by the *m*-hydroxydiphenyl assay (3). The samples are as follows: (A) *R. leguminosarum* 128C53; (B) *R. trifolii* 0403; (C) *R. leguminosarum* 128C63; (D) *R. trifolii* 162S7. In (A), the 0.05 absorbance scale is for the uronic acid assay.

strain elutes before the LPS1 fractions from the other *Rhizobium* strains, indicating that it has a larger molecular weight. The LPS1 fractions from *R. leguminosarum* 128C63, *R. trifolii* 0403, and *R. phaseoli* 127K17 (data not shown) all have the same elution volume, indicating that they are of a similar molecular weight. The LPS2 fractions from these four strains have the same elution volume on G-50 and also when rechromatographed on G-25. Standardization of the G-25 column with stachyose, maltotriose, lactose, and glucose show that all of the LPS2 fractions have a molecular weight of ca. 600. The polysaccharides released from *R. trifolii* 162S7 LPS elute as a broad hexose peak and as a uronic acid-rich peak. The uronic acid-rich peak has the same elution volume as the LPS2 fractions from the other *Rhizobium* strains. The broadness of the hexose peak indicates that it is very heterogeneous in size.

The presence of two polysaccharide fractions produced by mild acid hydrolysis (Fig. 1A to C) is a general phenomenon. We have obtained similar results for the LPSs from 4 other *R. leguminosarum* strains, 10 other *R. trifolii* strains, and 1 other *R. phaseoli* strain (unpublished data). Planque et al. (17) report the production of three polysaccharides, PS1, PS2, and PS3, on mild acid hydrolysis of an *R. leguminosarum* LPS. The PS1 fraction elutes at the G-50 void volume and is rich in glucose. We have observed a glucose-rich polysaccharide fraction eluting near the void volume when the LPS fraction is contaminated by residual extracellular or capsular polysaccharide. It is also possible that the glucose-rich PS1 peak observed by Planque et al. is due to the presence of a relatively small-molecular-weight glucan in their LPS preparation. Sepharose 4B gel filtration chromatography in an EDTA-triethylamine buffer separates the LPSs from residual CPSs, extracellular polysaccharides, and the small-molecular-weight glucan (5, 6). Planque et al. did not use this procedure when purifying their LPSs. In the case of *R. leguminosarum* 128C53 we have identified the small-molecular-weight glucan as a two-linked glucan (unpublished data).

The relative sugar compositions of the LPS1 and LPS2 fractions are given in Table 1. The LPS1 compositions vary greatly and reflect the variability of the intact LPSs previously reported (4–6, 27). All of the LPS1 fractions contain uronic acid and heptose, except the LPS1 from *R. leguminosarum* 128C53, which does not contain either of these sugars. All of the LPS2 fractions are rich in uronic acid, and in the case of *R. leguminosarum* 128C53, the uronic acid has been identified as galacturonic acid (5). Unlike the LPS1 fractions, all of the LPS2 fractions, except for LPS2 from *R. phaseoli* 127K17, are very similar in composition. The LPS2 from *R. phaseoli* 127K17 is different in that it contains glucose. None of the LPS1 fractions contain detectable levels of KDO. All of the LPS2 fractions from the G-50 column contained significant amounts of KDO; however, when purified further by G-25 column chromatography, all KDO elutes from the column at an elution volume which is identical to that of the KDO monomer.

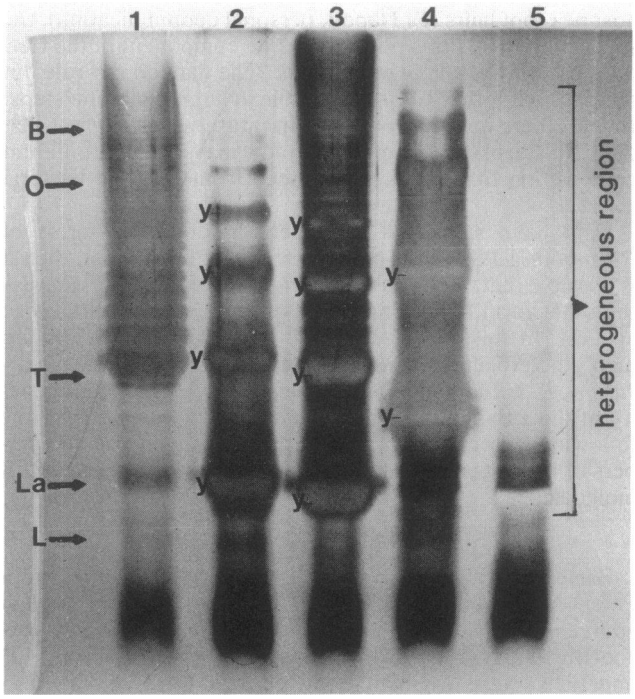

FIG. 2. Gel electrophoresis of *Rhizobium* LPSs. The gel (12% acrylamide) was run and stained as described by Hitchcock and Brown (10). The well samples were as follows: 1, 30 μg of *R. leguminosarum* 128C53 LPS; 2, 20 μg of *R. leguminosarum* 128C63 LPS; 3, 20 μg of *R. trifolii* 0403 LPS; 4, 20 μg of *R. trifolii* 162S7 LPS; and 5, 30 μg of *R. phaseoli* 127K14 LPS. The standards and molecular weights were: B, BSA (66,000); O, ovalbumin (45,000); T, trypsinogen (24,000); La, beta-lactoglobulin (18,400); and L, lysozyme (14,300). The proteins were stained as described by Fairbanks et al. (8). The proteins were not stained by the silver-staining technique used for LPSs (10). Bands marked by y are yellow-orange in color.

PAGE of *Rhizobium* LPSs. The LPSs were analyzed by polyacrylamide gel electrophoresis (PAGE) (Fig. 2). Each LPS shows many bands. These bands can be separated into two banding regions, a heterogeneous banding region containing many bands ranging in electrophoretic mobility from

TABLE 1. Composition of the LPS1 and LPS2 fractions derived from *Rhizobium* LPSs by mild acid hydrolysis

Strain	Composition of fractions[a]														
	LPS1											LPS2			
	Rha	Fuc	Man	Gal	Glc	UA	2OM6D	23OM6D	3NM3A6D	2A26D	Hep	Man	Gal	Glc	UA
R. leguminosarum 128C53	37	40	25	Tr	0	0	0	0	0	0	0	11	12	0	77
R. leguminosarum 128C63	0	16	Tr	Tr	5	33	0	5	17	0	24	17	14	0	67
R. trifolii 0403	4	13	7	Tr	4	27	8	0	15	0	21	18	14	0	67
R. phaseoli 127K17	0	21	Tr	Tr	10	37	0	0	26	6	0	18	17	15	50

[a] The relative percent sugar compositions are given: Tr, trace amounts present; Rha, rhamnose; Fuc, fucose; Man, mannose, Gal, galactose; Glc, glucose; UA, uronic acid; 2OM6D, 2-*O*-methyl-6-deoxyhexose; 23OM6D, 2,3-di-*O*-methyl-6-deoxyhexose; 3NM3A6D, 3-*N*-methyl-3-amino-3,6-dideoxyhexose; 2A26D, 2-amino-2,6-dideoxyhexose; Hep, heptose. The compositions of the intact LPSs from these *Rhizobium* strains have been presented in a previous publication (6). The LPS2 fractions were further purified by Sephadex G-25 chromatography before analysis.

that of bovine serum albumin (BSA) to that of beta-lactoglobulin and a lower-molecular-weight band which has an electrophoretic mobility greater than that of lysozyme. The banding patterns of the heterogeneous regions vary among the different LPSs; however, the lower-molecular-weight bands have the same electrophoretic mobility (greater than that of lysozyme) for all *Rhizobium* LPSs examined.

The LPS from *R. leguminosarum* 128C53 has a heterogeneous banding region with an electrophoretic mobility ranging from that of BSA to that of trypsinogen. This region consists of many bands which are separated from one another by a small, constant-molecular-weight factor. This pattern is very similar to that obtained for the LPSs from *Escherichia coli* and *Salmonella* strains (9, 10, 16). In the case of *E. coli* and *Salmonella* LPSs, the heterogeneous banding region consists of many different LPS molecules which are separated from one another by one O-antigen repeating unit, and the lower-molecular-weight band is due to incomplete LPSs which either lack the entire O-antigen repeating unit or contain only one or two repeating units (9, 10, 16). The *R. leguminosarum* 128C53 LPS also contains a single band with a mobility of beta-lactoglobulin.

The banding patterns of the heterogeneous region for the LPSs from *R. leguminosarum* 128C63 and *R. trifolii* 0403 are quite similar to one another but different from that of the other *Rhizobium* LPSs. For these two LPSs, the bands range in electrophoretic mobility from that of BSA to that of beta-lactoglobulin. This indicates that the LPSs for these two strains may be of a lower molecular weight than is the *R. leguminosarum* 128C53 LPS. In addition, the bands for these two LPSs are separated by a large-molecular-weight interval, possibly due to different aggregation states of the LPS molecule. There do not appear to be many bands separated by a small-molecular-weight interval as is the case for *R. leguminosarum* 128C53 LPS. The possibility that these LPSs are of a lower molecular weight than *R. leguminosarum* 128C53 LPS is supported by the fact that the LPS1 polysaccharides from *R. leguminosarum* 128C63 and *R. trifolii* 0403 elute at a significantly greater volume from the G-50 column than does the LPS1 from *R. leguminosarum* 128C53 (Fig. 1).

The heterogeneous banding region for the LPS from *R. trifolii* 162S7 also ranges in electrophoretic mobility from that of BSA to that of beta-lactoglobulin. However, this banding pattern appears to be a continuous staining region, and it is difficult to distinguish individual bands. There are, however, two distinct yellow bands present. One of these bands has a mobility between that of trypsinogen and that of beta-lactoglobulin, whereas the other band has a mobility between that of ovalbumin and that of trypsinosgen. It is not known what causes the color in these bands or in bands from the other *Rhizobium* LPSs. The high degree of heterogeneity of this LPS is also reflected in the broad G-50 hexose peak resulting from mild acid hydrolysis (Fig. 1).

The heterogeneous banding region for *R. phaseoli* 127K14 LPS shows approximately three bands with electrophoretic mobilities in the same region of that of beta-lactoglobulin. This LPS does not have the larger-molecular-weight bands which are present in the other LPSs.

To determine the relationship between the LPS bands observed by PAGE and LPS1 and LPS2 released by mild acid hydrolysis, the LPS from *R. leguminosarum* 128C53 was subjected to PAGE. Bands were cut from the gel corresponding to the heterogeneous region and to the lower-molecular-weight band, and their sugar compositions were determined (Fig. 3). The heterogeneous banding region contains all of the LPS sugars, whereas the lower-molecular-weight band contains only uronic acid and KDO. Thus it appears likely that LPS1 (Fig. 1 and Table 1) arises from the heterogeneous form of the LPSs, whereas LPS2 arises from the lower-molecular-weight LPSs. We did not detect galactose or mannose in the lower-molecular-weight LPS band. It is possible that these sugars were below detectable levels. It is difficult to obtain enough material from the gel for sugar analysis. In addition, there is, on occasion, some interference in acetylating the sugars, presumably from material arising from the polyacrylamide gel.

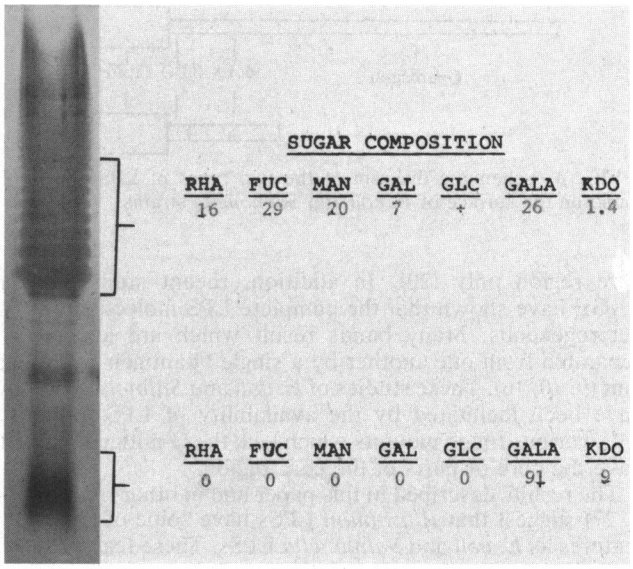

FIG. 3. Sugar compositions of the upper and lower banding regions of *R. leguminosarum* 128C53 LPS. The compositions are given as relative percent. Polysaccharide bands are not visible when electrophoresis of the mild acid-released polysaccharides is performed. RHA, Rhamnose; FUC, fucose; MAN, mannose; GAL, galactose; GLC, glucose; GALA, galacturonic acid; and KDO, 2-keto-3-deoxyoctonic acid.

DISCUSSION

The results described in this report show that *Rhizobium* LPSs are heterogeneous molecules. In addition to the various LPSs discussed in this paper we have obtained similar results for 4 other *R. leguminosarum* strains, 10 other *R. trifolii* strains, and 1 other *R. phaseoli* strain. All of these LPSs release two polysaccharides (LPS1 and LPS2) on mild acid hydrolysis, and the majority give banding patterns determined by PAGE similar to those of 0403 and *R. leguminosarum* 128C63 LPSs (Fig. 2).

At the present time, the causes for the heterogeneity in *Rhizobium* LPSs are not understood. However, several possibilities can be suggested by comparing the results with those of published studies on the LPSs from *E. coli* and *Salmonella* strains. The LPSs from *E. coli* and *Salmonella* strains have been shown to be present in two general forms (Fig. 4). One form is a complete molecule consisting of the lipid-A, core oligosaccharide, and repeating oligosaccharide known as the O-antigen. The other form is the incomplete molecule which lacks the O-antigen (12). The polysaccharide portion of the LPS is attached to the lipid-A via an acid-labile ketosidic bond with KDO (12, 20). Mild acid hydrolysis releases a polysaccharide consisting of the O-antigen attached to the core region and an oligosaccharide which is the

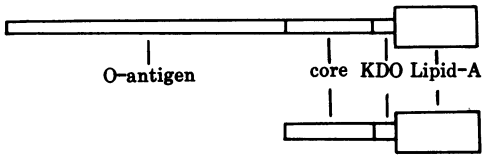

FIG. 4. Schematic diagram of the two types of LPS generally found on the surface of *E. coli* and *Salmonella* strains.

core region only (20). In addition, recent studies using PAGE have shown that the complete LPS molecule is very heterogeneous. Many bands result which are apparently separated from one another by a single O-antigen repeating unit (9, 10, 16). These studies of *E. coli* and *Salmonella* LPSs have been facilitated by the availability of LPS mutants, e.g., various rough mutants which lack the O-antigen but still have the core or parts of the core region.

The results described in this paper and in other reports (4–6, 27) suggest that *Rhizobium* LPSs have some of the same features as *E. coli* and *Salmonella* LPSs. These features are: (i) *Rhizobium* LPSs contain lipid-A, KDO, and polysaccharide, and (ii) the lipid-A–polysaccharide bond can be hydrolyzed by mild acid. However, our results also suggest that *Rhizobium* LPSs differ in several ways from *E. coli* and *Salmonella* LPSs. These differences are discussed below.

***R. leguminosarum* 128C53 LPS.** The results determined by PAGE for this LPS are similar to those reported for *E. coli* and *Salmonella* LPSs and suggest that the heterogeneous banding region consists of complete LPS molecules which are separated from one another by one O-antigen repeating unit and that the lower-molecular-weight band is the LPS which lacks the O-antigen repeating unit. This seems to be supported by the composition analysis of the heterogeneous region and the lower-molecular-weight band (Fig. 3) and suggests that the complete LPS molecule consists of a rhamnose-fucose-mannose O-antigen and a galacturonic acid-rich core. If this is true then one would expect, as with *E. coli* and *Salmonella* LPSs, that the LPS1 and LPS2 fractions released by mild acid hydrolysis would consist of the O-antigen-core polysaccharide and the core oligosaccharide, respectively. However, LPS1 from *R. leguminosarum* 128C53 does not contain galacturonic acid, the major sugar found in LPS2 (Table 1). In addition, neither LPS1 nor LPS2 contain KDO. All detectable KDO, after mild acid hydrolysis, elutes as monomeric KDO. These anomalies in comparison with characteristics of *E. coli* and *Salmonella* LPSs suggest several possibilities. (i) The presence of only monomeric KDO after mild acid hydrolysis may be due to a KDO residue which has an acid-labile bond on its nonreducing side. This could be due to the presence of a KDO di- or trisaccharide as in *E. coli* or *Salmonella* LPS. The fact that KDO is not detected in LPS1 or LPS2 may be because it is either present at below detectable levels or present in a form which no longer reacts colorimetrically in the thiobarbituric acid assay. The release of monomeric KDO and its absence in LPS1 and LPS2 appears to be a general feature of all LPSs described in this report. (ii) The absence of galacturonic acid (the major LPS2 sugar) in the LPS1 fraction may be due to the presence of an acid-labile linkage between the O-antigen and the core region which is hydrolyzed by 1% acetic acid. The absence of core sugars in the O-antigen polysaccharide released by mild acid hydrolysis has also been observed for the LPSs from *Serratia marcescens* Bizo and *S. marcescens* 08 (21, 23). A mild acid-labile O-antigen-core bound has been suggested for these LPSs (26).

LPSs from *R. trifolii* 0403 and *R. leguminosarum*. The banding patterns, determined by PAGE, of these LPSs are similar to one another but quite different from the other *Rhizobium* LPSs (Fig. 2) and from *E. coli* and *Salmonella* LPSs. The heterogeneous region for these two LPSs does not show many bands separated by a small-molecular-weight interval as is the case for *R. leguminosarum* 128C53 LPS and *E. coli* and *Salmonella* LPSs. Instead, there are several major bands separated by a large-molecular-weight interval, with the lowest-molecular-weight band (of the heterogeneous region) being the major band. If the bands of the heterogeneous region of *R. leguminosarum* 128C53 LPS represent various LPS molecules separated from one another by one O-antigen repeating unit, then the absence of this banding pattern suggests that this type of variability does not exist for *R. leguminosarum* 128C63 and *R. trifolii* 0403 LPSs. The higher electrophoretic mobility of these LPSs and also the relatively higher elution volume of the LPS1 fractions compared with those of *R. leguminosarum* 128C53 suggest that the polysaccharide chain of the complete *R. leguminosarum* 128C63 and *R. trifolii* 0403 LPSs may be relatively short. A possible explanation for these differences is that the complete form of the LPSs from these two strains does not have an O-antigen consisting of an oligosaccharide repeating unit but consists of a complex oligosaccharide attached to a uronic acid-rich core region. In this case the LPSs would resemble the LPSs from semirough mutants of the genus *Salmonella* which contain only one O-antigen repeating unit attached to the core region (for review, see reference 26).

LPS from *R. phaseoli*. The heterogeneous banding region observed by PAGE consists of a few bands with an electrophoretic mobility near that of beta-lactoglobulin (Fig. 2; see above). Higher-molecular-weight bands, which are present in the other *Rhizobium* LPSs, are absent. This would suggest that *R. phaseoli* 127K14 LPS, unlike *R. leguminosarum* 128C53 LPS, does not consist of many LPS molecules which vary in the number of O-antigen repeating units. Thus, as for *R. leguminosarum* 128C63 and *R. trifolii* 0403 LPSs, it is possible that some *R. phaseoli* LPS also consist of a uronic acid-rich core to which is attached either a complex oligosaccharide or a very short O-antigen. The reason for the absence of several higher-molecular-weight *R. phaseoli* 127K14 LPS bands is not known; however, it may reflect a difference in the type of lipid-A compared with the other *Rhizobium* LPSs or a difference in the core oligosaccharide. In fact, the LPS2 oligosaccharide from *R. phaseoli* 127K17 LPS is different from the other LPS2 fractions in that it contains glucose (Table 1).

LPS from *R. trifolii* 162S7. The properties of this LPS are different from those of the other *Rhizobium* LPSs in several ways. Mild acid hydrolysis and G-50 chromatography give a uronic acid-rich peak with an elution volume similar to that of LPS2 from the other *Rhizobium* strains; however, instead of a symmetrical peak similar to that of LPS1, a broad hexose peak results, indicating a great deal of heterogeneity in size. The reason for this heterogeneity is not known; however, it could be due to the presence of a mild acid-labile bond within the LPS1 polysaccharide or to a great deal of variability in the chain length of this polysaccharide. The heterogeneity of LPS1 is also reflected in the PAGE results which show an almost continuous staining region ranging in electrophoretic mobility from that of lysozyme to that of BSA.

Conclusions. Previous reports have shown that *Rhizobium* LPSs possess a great deal of structural variability, even among strains of a single species (4–6, 27). In this report,

data were presented which show that *Rhizobium* LPSs are highly heterogeneous molecules. This complexity of *Rhizobium* LPSs makes their characterization somewhat difficult, but it is this complexity which provides these molecules with a great potential for determining the interaction between a *Rhizobium* organism and another organism, including the legume host.

The role of *Rhizobium* LPSs in symbiosis is not known. Analysis of the LPSs from specific symbiotic mutants is in progress to determine what role, if any, the LPSs may play in symbiosis. It also would be useful to have *Rhizobium* mutants which are altered in their LPSs and to then determine the effect of these alteration(s) on the symbiotic process.

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