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A Lipopolysaccharide Mutant of *Bradyrhizobium japonicum* that Uncouples Plant from Bacterial Differentiation

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The Tn*5*-containing fragment from a non-nodulating mutant of *Bradyrhizobium japonicum*, strain ML142, was introduced into *B. japonicum* strain 61A101c by marker exchange to construct strain JS314. Strain JS314 failed to nodulate several soybean varieties tested. However, on a few varieties nodulelike structures were induced to a frequency of 54% of the plants inoculated. The ultrastructure of these nodules was studied in detail by light and electron microscopy. The nodules were devoid of internal bacteria, possessed central vascular tissue (unlike the lateral vascular tissue of a normal nodule), and exhibited localized cell death of epidermal cells. Study of the cell surface polysaccharides of strain JS314 revealed that the exopolysaccharide of this strain was identical to that of the wild type. However, the lipopolysaccharide (LPS) of strain JS314 showed gross differences from that isolated from the wild-type strain. Specifically, the LPS of strain JS314 appeared to lack the high molecular weight LPS I form, strongly suggesting that the LPS lacks the *O*-chain. Glycosyl-composition analysis showed that the LPS of mutant JS314 lacked 2,3-di-*O*-methylrhamnose, 3-*O*-methylrhamnose, fucose, and quinovosamine. These results indicate that LPS I in *B. japonicum* is essential for bacterial infection of soybean, but is not required to initiate plant cortical cell division, an early plant response to infection.

Additional keywords: nitrogen fixation, *Rhizobium*.

Rhizobium/*Bradyrhizobium* infection of leguminous plants is a complex, multistep process initiated by the attachment of a compatible rhizobium to the host plant root-hair cell. Subsequent events then lead to a curling of the root-hair cell, penetration of the cell by the rhizobia, and the formation of an infection thread, the route by which the rhizobia gain access to the root cortex. The final product of this process is a nitrogen-fixing root nodule, a morphologically and developmentally distinct plant organ. Two basic types of nodules are formed depending on plant species, determinate nodules formed on plants such as bean and soybean (*Glycine max* (L.) Merr.), and indeterminate nodules formed on plants such as alfalfa, pea, and clover. In a recent review, Kijne (in press) pointed out that the infection threads of these two types are distinct. Determinate nodules possess narrow infection threads containing little internal matrix material with the bacteria usually in single file and in close contact with the infection thread membrane. In contrast, indeterminate nodules possess broad infection threads containing considerable matrix material and several bacteria abreast within the thread.

Considerable evidence exists indicating that the cell surface polysaccharides of *Rhizobium*/*Bradyrhizobium* species are essential for infection. A compelling case can be made for an essential role in nodulation for the exopolysaccharide (EPS) of those *Rhizobium* species nodulating plants that form indeterminate, broad infection thread nodules. EPS-deficient (Exo$^-$) mutants of *Rhizobium leguminosarum* bv. *viciae* Jordan and *R. l.* bv. *trifolii* Jordan, as well as *R. meliloti* Dangeard, do not infect their host species and do not form infection threads (Borthakur *et al.* 1986; Diebold and Noel 1989; Finan *et al.* 1985; Leigh *et al.* 1987; Leigh *et al.* 1985; Napoli and Albersheim 1980) The best studied examples are the *exo* mutants of *R. meliloti* that form empty, pseudonodules on alfalfa (e.g., Leigh *et al.* 1987; Finan *et al.* 1985; Leigh *et al.* 1985; Long *et al.* 1988). These pseudonodules are exemplified by a central vascular tissue distinctly different from the lateral vascular channels found in functional nodules. Interestingly, the overall structure of the EPS does not appear critical for infection of all host plant species because the production of a second, structurally distinct EPS suppresses the symbiotic defect of these *R. meliloti exo* mutants (Glazebrook and Walker 1989; Zhan *et al.* 1989). Diebold and Noel (1989) suggested that the role of EPS in the formation of narrow vs broad infection thread nodules may differ. They found that Tn*5*-induced Exo$^-$ mutants of *R. l.* bv. *phaseoli* Jordan were fully proficient at determinate nodulation of bean. However, these same mutations, when transferred to *R. l.* bv. *viciae* or *R. l.* bv. *trifolii*, resulted in an Exo$^-$ phenotype and the formation of empty, pseudonodules on their respective hosts, pea and clover. The implication of this work is that EPS is essential for the infection and formation of the broad infection thread-type nodule (e.g., by supplying components of the infection thread matrix), but is not required for the formation of the narrow infection thread-type nodule. Further support for this hypothesis comes

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from studies of Exo$^-$ mutants of *B. japonicum* that form effective, determinate nodules on soybean (Law *et al.* 1982).

In contrast to EPS, an intact lipopolysaccharide (LPS) appears essential for the infection of narrow infection thread-type nodules (e.g., Diebold and Noel 1989; Noel *et al.* 1986; Stacey *et al.* 1984; VandenBosch *et al.* 1985). Noel *et al.* (1986) isolated two Tn*5*-induced mutants of *R. l.* bv. *phaseoli* defective in LPS synthesis. These mutants infected bean but formed wide, abnormal infection threads that aborted early. The nodules formed were abnormal in containing a central vascular tissue. Previously, we reported on an ultraviolet light-induced mutant of *B. japonicum* that was defective in LPS synthesis and was unable to nodulate soybean (Stacey *et al.* 1984). In this case, the mutant was competent in attachment to soybean roots and in the induction of root-hair curling, but failed to penetrate the root or cause the induction of infection threads or pseudonodules. In contrast to these results, LPS-deficient mutants of rhizobia that form indeterminate nodules are fully functional in formation of infection threads but may form ineffective (i.e., incapable of fixing nitrogen) nodules. For example, *R. l.* bv. *viciae* mutants altered in LPS structure are impaired in release from the infection thread (De Maagd *et al.* 1989) or degenerate rapidly after release (Priefer *et al.* 1989).

In this report, we provide further support for the hypothesis that an intact LPS is essential for the formation of determinate nodules. Moreover, we describe a mutant of *B. japonicum* that phenotypically resembles the previously described Exo$^-$ mutants of *Rhizobium* species incapable of infection thread formation but inducing empty pseudonodules. These mutants provide evidence that an intact LPS is essential during the earliest stages of soybean root-hair penetration and indicate that the induction of nodule formation on soybean can be uncoupled from bacterial infection.

MATERIALS AND METHODS

Bacterial strains and plasmids. *B. japonicum* strains were grown at 30° C on YEM medium (Wacek and Brill 1976), RDY medium (Bishop *et al.* 1976), YS medium (RDY medium without gluconate and glutamate), or defined medium (Mort and Bauer 1980). *Escherichia coli* strains were grown at 37° C in Luria broth (Davis *et al.* 1980). Bacterial strains and plasmids used in this study are listed in Table 1. Antibiotics were added to the medium to the final concentrations of: *B. japonicum*, 100 μg/ml of kanamycin (Km) and 200 μg/ml of streptomycin (Sm); and *E. coli*, 50 μg/ml of ampicillin (Ap), 15 μg/ml of tetracycline (Tc), 30 μg/ml of chloramphenicol (Cm), and 50 μg/ml of Km.

Recombinant DNA methods. Standard DNA manipulations such as restriction digestion of DNA, agarose gel electrophoresis, Southern blot hybridization, and preparation of radiolabeled DNA were performed as described by Sambrook *et al.* (1989) or as described previously (So *et al.* 1987). A total genomic DNA library of *B. japonicum* strain ML142 was constructed by digestion with *Eco*RI and cloning into the cosmid vector pHC79 (Hohn 1979) as described by So *et al.* (1987). The Tn*5*-containing clones were selected by resistance to Tc (encoded by pHC79) and Km (encoded by Tn*5*).

Homogenotization. The cloned Tn*5*-mutated DNA was introduced into wild-type *B. japonicum* strain 61A101c by triparental mating involving a helper plasmid pRK2073 (Ruvkin and Ausubel 1981; Ditta *et al.* 1980), using YS medium. Marker exchange was selected by resistance to Km and Sm encoded by Tn*5*. Successful replacement events were confirmed by Southern blot analysis of genomic DNA.

Nodulation assay. Soybean seeds were surface-sterilized, germinated, and inoculated (Russell *et al.* 1985). Root-hair curling was examined as described by Halverson and Stacey (1985). Nitrogen-fixing ability of the nodules was tested by the acetylene reduction assay (Wacek and Brill 1976). Bacteria were isolated from soybean nodules by the procedure of Vincent (1970), and the bacteria's identity was confirmed by plating onto the appropriate selective medium and by reinoculation of the appropriate host.

Purification of bacterial polysaccharides. Bacterial EPSs were purified from bacteria-free defined medium by ethanol precipitation (Carlson 1982). The precipitate was dissolved in deionized water, dialyzed extensively, and lyophilized.

Cells were grown on YEM or RDY medium, and the LPS was extracted (Westphal and Jann 1965). Bacterial LPSs were purified from the phenol layer of the phenol/water extract as previously described for *B. japonicum* LPS (Carrion *et al.* 1990). Briefly, the phenol layer was dialyzed against deionized water and cellular debris was removed by centrifugation; the supernatant was treated with proteinase K, ribonuclease, and deoxyribonuclease, and was dialyzed and lyophilized. The LPS was further purified by gel-filtration chromatography or by ultracentrifugation at 100,000 × *g* for 4 hr.

Polyacrylamide gel electrophoresis (PAGE) analysis. PAGE analysis was performed with deoxycholate as the detergent in place of sodium dodecyl sulfate (SDS) as previously described (Krauss *et al.* 1988). The gels were silver-stained according to the method of Hitchcock and Brown (1983).

Table 1. Bacterial strains and plasmids used in this study

Designation	Characteristics[a]	Reference or source
Bradyrhizobium japonicum		
61A101c	Wild type	LiphaTech, Inc.[b]
ML142	61A101c::Tn*5*, Nod$^-$	So *et al.* 1987
JS314	61A101c::Tn*5*, Nod$^-$	This study
Escherichia coli		
HB101	F$^-$, *hsdR hsdM recA thi-1 leuB6 proA2 lacZ4 supE44*	Davis *et al.* 1980
DH1	F$^-$ *recA endA1 gyrA96 thi-1 hsdR17 supE44* λ$^-$	Sambrook *et al.* 1989
Plasmids		
pRK2073	*tra*$^+$ (RK2:ColE1)	Leong *et al.* 1982
pHC79	Tc^r Ap^r cosmid	Hohn 1979
pJS34, 35, 36, 313, 314, 315, 316	pHC79 clones containing 11.2-kb *Eco*RI fragment with Tn*5* insertion	This study

[a] Tc = tetracycline, Ap = ampicillin; ^{r} = resistant; and kb = kilobase.
[b] Milwaukee, WI.

Analytical techniques. Neutral sugars were analyzed by hydrolysis with 2 M trifluoroacetic acid (TFA), by reduction with $NaBD_4$, by acetylation with acetic anhydride in pyridine, and by analysis with gas chromatography (GC) and combined GC-mass spectrometry (GC-MS) (York *et al.* 1985). Analysis by GC was performed by using a 30-m fused silica SP2330 capillary column (Supelco, Inc., Bellefonte, PA). Amino sugars were identified and quantified by hydrolysis in 4 M HCl for 16 hr at 100° C, N-acetylated, reduced, acetylated, and analyzed by GC and GC-MS as described above. Identification and quantification were accomplished by comparison with authentic standards and by mass spectroscopy. Further details are described in Table 3 below. Uronic acids were quantified by the method of Blumenkrantz and Asboe-Hansen (1973) and 3-deoxy-D-manno-2-octulosonic acid by the method of Weissbach and Hurwitz (1958). Fatty acids were determined by methanolysis and GC-MS analysis of their methyl esters (Weisshaar and Lingens 1983). GC analyses of the amino-sugar alditol acetates and fatty acid methyl esters were performed with a 30-m DB-1 capillary column (J&W Scientific, Folsom, CA).

Nuclear magnetic resonance (NMR) spectroscopy was accomplished with a Bruker AM250 instrument. The sample (2–5 mg) was dissolved in D_2O and lyophilized. This step was repeated, and the sample was dissolved in D_2O and analyzed. The spectra were run at 300° K, and all chemical shifts are relative to TSP (3-[trimethylsilyl]propionic acid).

Electron microscopy. Transmission electron microscopy (TEM) of soybean roots and nodules was performed as previously described (Roth and Stacey 1989a,b).

Table 2. Nodulation pheotype of JS314 on different cultivars of *Glycine max*

Cultivar	Percentage of nodulated plants[a]	Nodule description[b]
BSR101	50	White, empty
Centry 84	30	White, empty
Clark 63	54	Small, empty
Clay	25	Small, empty
CN 290	0	
Dawson	0	
Douglas	10	Popcorn
Elf	0	
Essex	7	Popcorn
Fayette	0	
Gnome 85	50	White, empty
Hack	25	Small, empty
Harasoy 63	0	
Hodgson 78	33	White, empty
Hardee	0	
Pella	22	Popcorn
Peking	0	
Pershing	25	Popcorn
Pyramid	0	
Union	0	
Williams	0	

[a] Ten to 15 plants were inoculated with 1 ml of culture (approximately 10^8 cells per milliliter). Plant roots were examined for nodules 28 days postinoculation, and the N_2-fixing ability was assayed by acetylene reduction. None of the plants examined showed N_2-fixation activity. The results shown were consistent over two replications.

[b] Small = visible bumps on roots; white = structures removed from roots and sectioned to reveal white interior; popcorn = calluslike appearance with a fuzzy covering; empty = lacking bacteria as examined by light microscopy and, in a few cases, by transmission electron microscopy.

RESULTS

B. japonicum strain JS314, a Nod⁻ mutant. Previously, we reported the isolation of a variety of symbiotically defective mutants of *B. japonicum* after random Tn*5* mutagenesis (So *et al.* 1987). One such mutant from strain 61A101c, strain ML142, exhibited a Nod⁻ phenotype when tested on soybean (cv. Essex). To clone the mutated region from this strain, we constructed a genomic library and screened the clones for resistance to Tc (encoded by the vector pHC79) and Km (encoded by Tn*5*). In this way, a number of overlapping cosmid clones were isolated containing Tn*5* (i.e., pJS34, 35, 36, 313, 314, 315, and 316; Table 1). Subsequent hybridization of Tn*5* (i.e., pBR322::Tn*5*) to restricted DNA of these cosmid clones indicated that the insertion was present in a 11.2-kb *Eco*RI fragment (data not shown). This fragment was equivalent in size to the fragment detected by Tn*5* hybridization to genomic DNA of ML142. The previously reported estimation of this fragment as 12.7 kb (So *et al.* 1987) was incorrect.

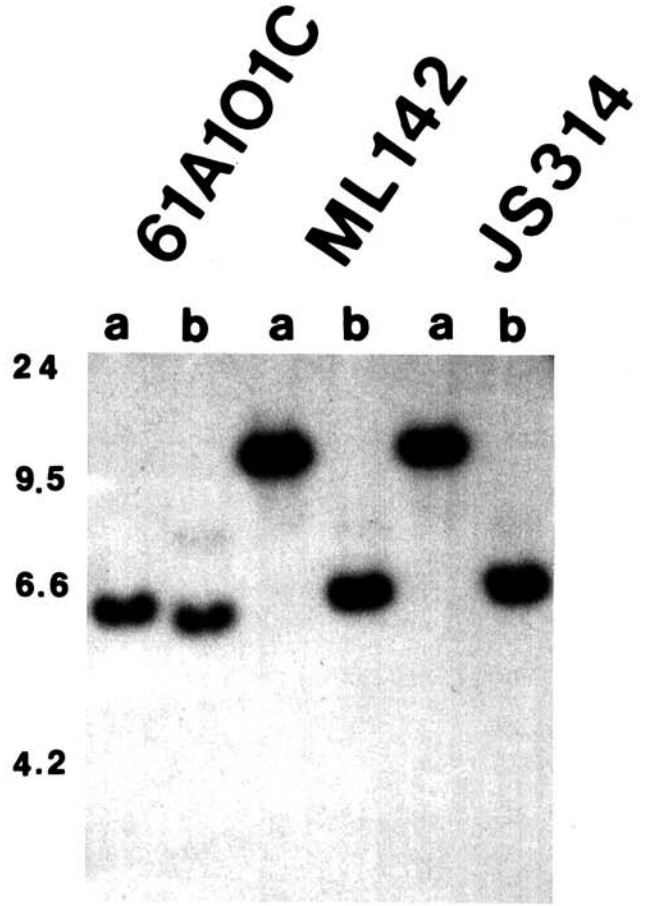

Fig. 1. Southern hybridization analysis of genomic DNA from wild-type strain 61A101c, mutant ML142, and mutant JS314. *Eco*RI digests (lane a) and *Bam*HI digests (lane b) of genomic DNA were probed with the nick-translated 11.2-kb *Eco*RI fragment containing Tn*5*. The hybridizing band in lane b of ML142 and JS314 is a doublet.

To determine whether the Tn*5* insertion in the cloned DNA was indeed responsible for the nodulation phenotype, the cloned DNA fragment containing Tn*5* (i.e., pJS314) was introduced into the wild-type strain 61A101c to induce homologous recombination and marker exchange. A successful replacement event was confirmed by Southern blot analysis of *Eco*RI and *Bam*HI digests of genomic DNA with the gel-purified 11.2-kb *Eco*RI fragment from pJS314 as probe (Fig. 1). Both strain ML142 (original mutant) and JS314 (homogenote) contained a single *Eco*RI fragment of 11.2 kb in size that hybridized to the probe. In the wild-type strain 61A101c, a 5.5-kb *Eco*RI fragment hybridized, into which the 5.7-kb Tn*5* was inserted in ML142 and JS314 to yield the 11.2-kb fragment detected.

The symbiotic phenotype of strain JS314 was determined by plant infection of soybean (cv. Essex). As shown in Figure 2, JS314 almost completely failed to nodulate soybean, indicating that the Tn*5* mutation was indeed the cause of the symbiotic phenotype. Coincident with these experiments, strain JS314 was inoculated onto a variety of other cultivars of soybean (Table 2). Surprisingly, the phenotype varied depending on the cultivar inoculated. Of the cultivars tested, 48% failed to nodulate, while on other cultivars 7–54% of the plants inoculated formed nodulelike structures. However, normal nodules were not formed on any of the cultivars examined, and nitrogenase activity was not detected.

Nodulelike structures formed by strain JS314. Microscopic examination of soybean roots after inoculation by strain JS314 indicated that normal root-hair curling occurred (i.e., as compared to strain 61A101c; data not shown). However, as indicated above, JS314 could induce the production of one or two anomalous protrusions on the primary roots of certain cultivars of soybean after a long delay (about 17 days postinoculation). These structures had a calluslike appearance with a fuzzy covering and were given the trivial, but descriptive name, "popcorn" nodules. As shown in Table 2, not all soybean cultivars inoculated showed this popcorn morphology with some simply giving small, white, empty nodules.

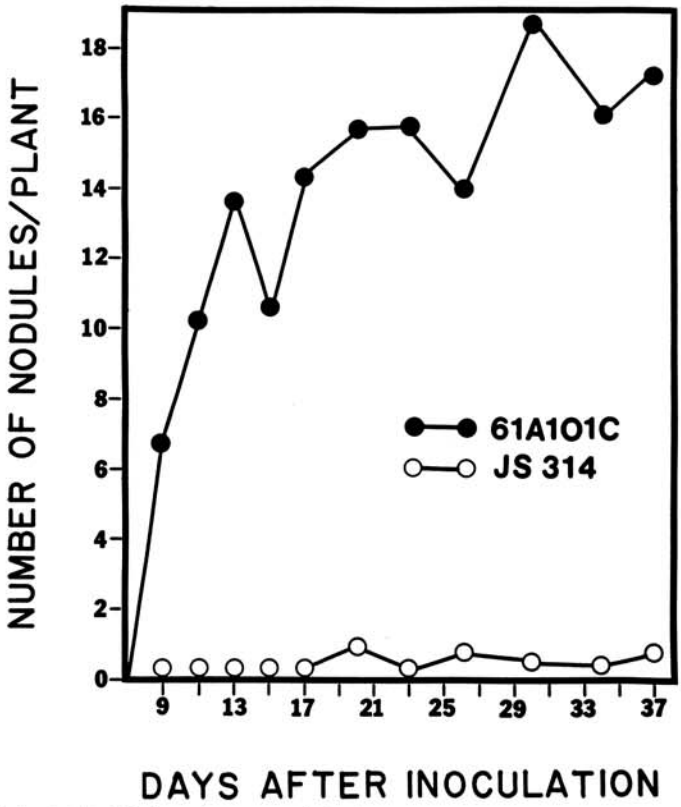

Fig. 2. Nodulation phenotype of the homogenote JS314 and the wild-type strain 61A101c. Two sets of 110 plants were inoculated with each strain (1 ml at approximately 10^8 cells per milliliter). At time intervals, 10 plants were sacrificed to determine the nodulation phenotype.

The popcorn nodules were analyzed further by light and transmission electron microscopy. Light microscopy revealed that they have a single meristematic area that tends to be somewhat depressed at its center (Fig. 3). Unlike normal nodules, a single vascular strand traverses the structure and is surrounded by several sclereid and many other cells (Fig. 4). TEM examination showed no evidence of infection threads or bacteria. Most plant cells contain a central vacuole typical of root cells and have a thin layer of peripheral cytoplasm that contains small numbers of the usual cell organelles (Fig. 5A). No cells were observed that are typical of normal nodule cells, which have no central vacuole and have large numbers of Golgi bodies, mitochondria, amyloplasts, as well as large amounts of endoplasmic reticulum (c.f., Roth and Stacey 1989a,b). The appearance of popcorn nodules is apparently due to the cytoplasmic invasion and degradation of the cell walls at intervals (Fig. 5B), to endocytosis of vesicles containing cell wall material (Fig. 5C), and to the formation of cell wall remnant leaflets (Fig. 5B). The cells subsequently die, leaving a layer of such remnants covering the nodule (Figs.

Table 3. Composition of the lipopolysaccharides from *Bradyrhizobium japonicum* 61A101c and the symbiotic mutant JS314

Glycosyl residue	61A101c[a]	JS314[a]
2,3-Di-*O*-methylrhamnose	25	ND
3-*O*-Methylrhamnose	3.1	ND
Fucose	7.9	ND
4-*O*-Methylmannose	5.6	15
Mannose	19	44
Glucose	6.9	18
Quinovosamine	17	ND
Fucosamine	Tr	Tr
2,3-Diamino-2,3-dideoxyglucose	14	10
Glucosamine	Tr	Tr
Uronic acid	ND	ND
2-Keto-3-deoxyoctonate	2.4	12
Fatty acyl residues[b]		
3-OH-12:0	6.8	4.9
16:0	13	3.5
3-OH-14:0	13	13
18:1	30	17
14:0	3.1	6.5
27-OH-28:0	34	56

[a]The glycosyl values are given as the percentage of the total carbohydrate, and account for 30 and 27% of the lipopolysaccharide mass for strains 61A101c and JS314, respectively. Quantitation of the methylated rhamnosyl residues was accomplished by using the detector response of standard rhamnose. Quantitation of all the aminosugars was accomplished by using the detector response of standard glucosamine. Inositol was added as the internal standard. ND = not detected; Tr = trace amount.

[b]The fatty acyl values are given as the percentage of total gas chromatography peak areas of all the fatty acyl components.

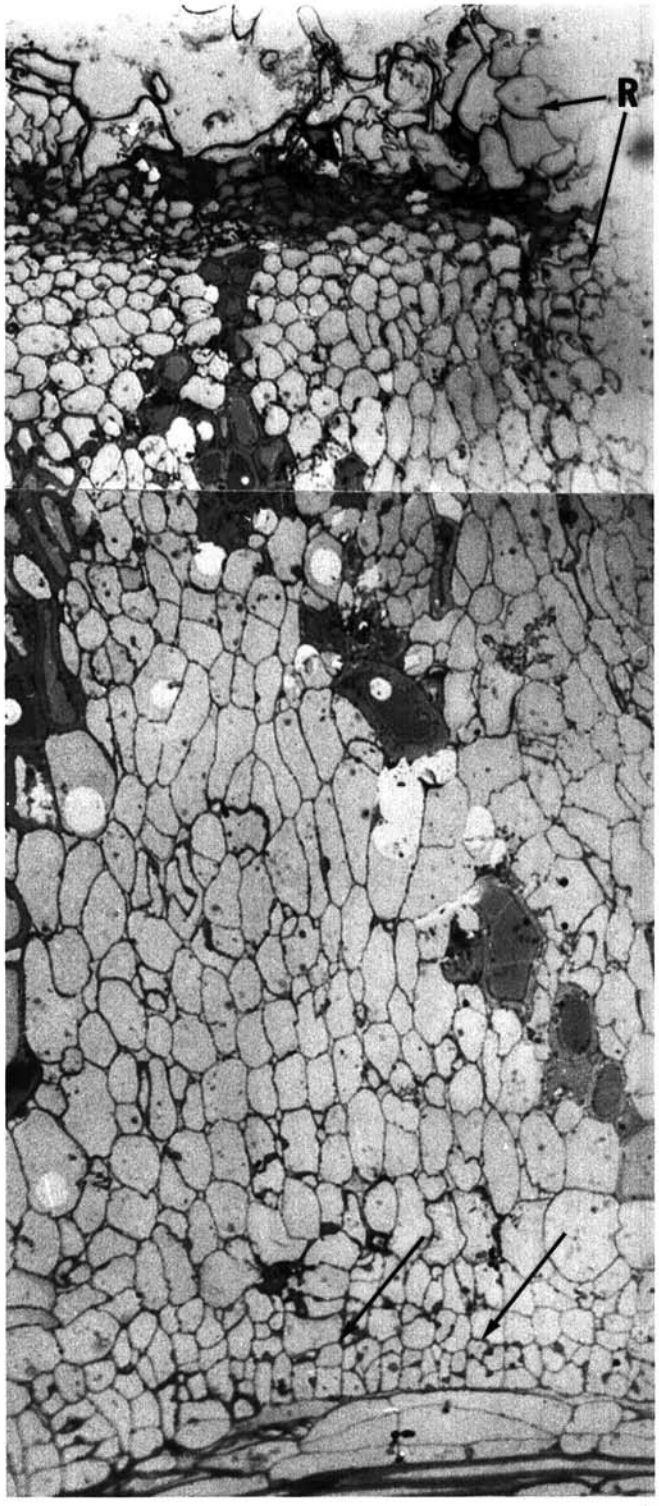

Fig. 3. Light micrograph of a plastic-embedded longitudinal section of nodulelike structures formed by JS314 on soybean cultivar Essex. Many cells have been induced to proliferate but are not organized into a typical nodule. The central area is composed of cells that have only a thin layer of cytoplasm. The cells near the surface eventually die, leaving behind remnants (R) of cell walls that are sloughed off around the edges. Near the root, at the bottom of the field, a double palisade of cells is often seen (arrows). Magnification: ×260.

3 and 4). At their tips, the popcorn nodules have a protruding ring of these leaflets, but at the center, the meristem precludes this action, so that the top has a depressed darkened center, hence the "popcorn" terminology (Fig. 3).

Cell surface characteristics of strain JS314. Strain JS314 exhibited no observable difference in colony morphology or mucoidy when compared to the wild type on a variety of media. Moreover, the growth rate of strain JS314 in minimal medium was indistinguishable from the wild type. Strain JS314 was also actively motile (i.e., by phase-contrast microscopy) and its subpolar flagellum could be detected by TEM (data not shown). However, JS314 cells in liquid medium aggregated to form sizeable clumps that were observed at all phases of growth. The latter observation indicated that the cell surface of strain JS314 is modified.

Characterization of EPS. Analysis of EPS from strain JS314 and the wild-type parent 61A101c indicated that they are identical in composition and in their glycosyl linkages. Both EPSs consist of rhamnose and 4-*O*-methylglucuronic acid in a 3:1 ratio. Methylation analysis revealed identical glycosyl linkages consisting of 3- and 4-linked rhamnosyl, 3,4-linked rhamnosyl and terminal 4-*O*-methylglucuronic acid. The identity of these two EPSs is reflected in their proton NMR spectra (Fig. 6). The resonances between δ1.2 and δ1.4 are due to the methyl protons of the rhamnosyl residues; the resonance at δ3.45 is due to the $-OCH_3$ group of the 4-*O*-methylglucuronic acid; the resonances between δ3.1 and δ4.3 are due to the ring protons of the glycosyl residues; those between δ4.4 and δ5.1 are due to the anomeric protons; and the intense resonance at about δ4.7 is due to water. The resonance at δ2.2 is due to a nonEPS component that was largely, but not completely, removed by gel-filtration chromatography. This component was present in both EPS preparations.

Characterization of the LPS. Analysis of the parent and mutant LPS by PAGE showed that the mutant LPS lacks the higher molecular weight form of the LPS, LPS I, and contains only the lower molecular weight LPS, LPS II (Fig. 7). Previous results for both *R. leguminosarum* and

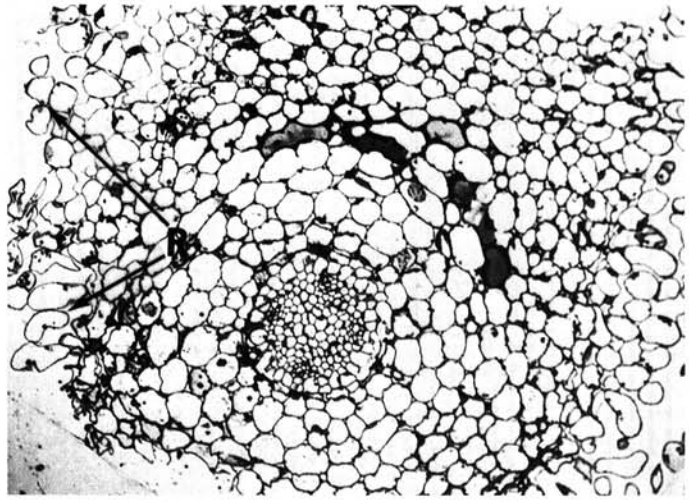

Fig. 4. Cross-section of a nodule. In the center, a primitive vascular system is found, while peripherally cell remnants (R) are present in large numbers. Magnification: ×260.

B. japonicum LPSs have shown that LPS I is the complete form of the LPS in that it contains the lipid A, core oligosaccharides, and *O*-chain, while LPS II is incomplete in that it lacks the *O*-chain (Carrion *et al.* 1990; Carlson 1984; Carlson *et al.* 1987; Brink *et al.* 1990; Carlson and Lee 1983; Puvanesarajah *et al.* 1987). Therefore, these

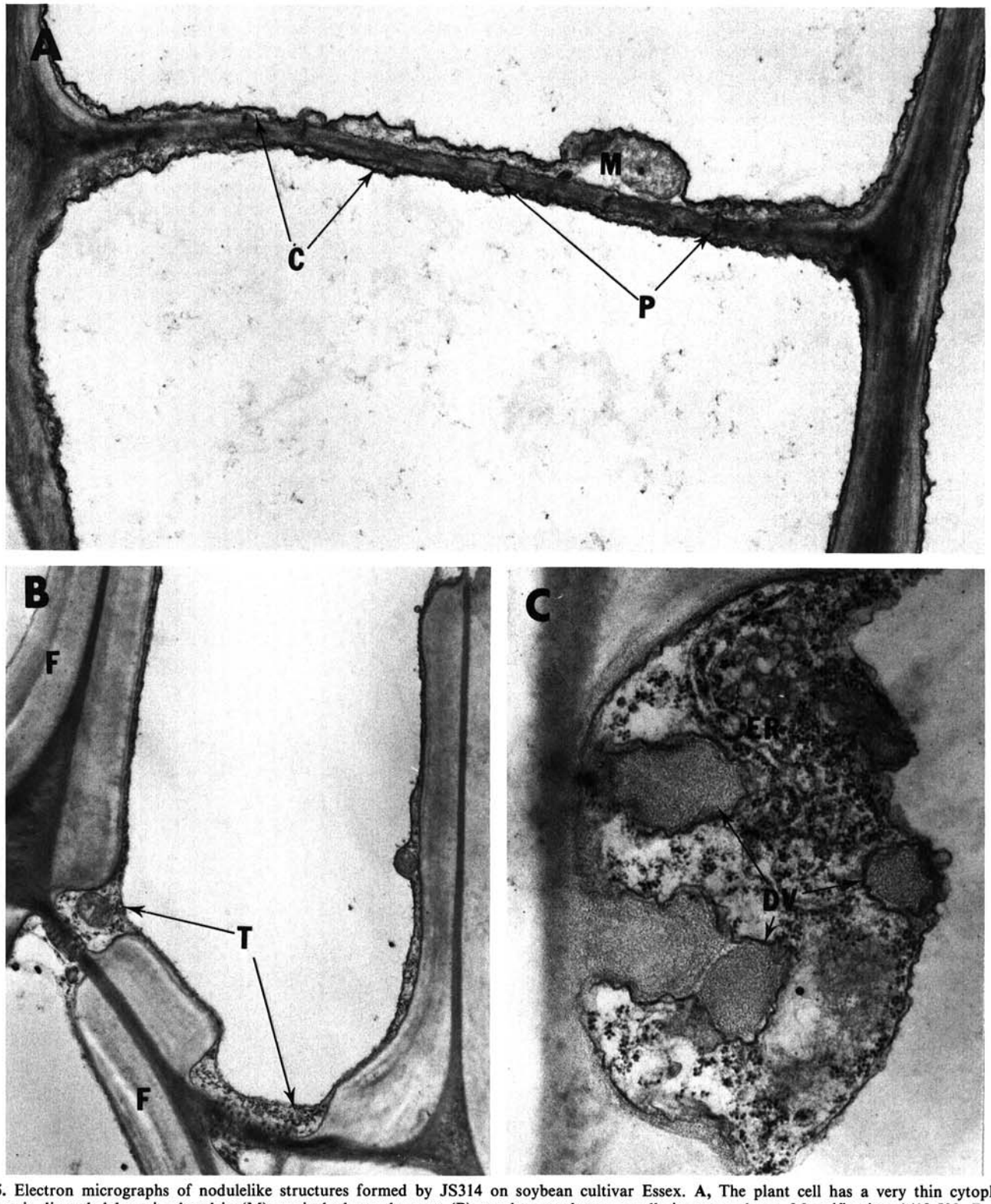

Fig. 5. Electron micrographs of nodulelike structures formed by JS314 on soybean cultivar Essex. **A,** The plant cell has a very thin cytoplasm (C) that is distended by mitochondria (M); typical plasmodesmata (P) can be seen between cells in some places. Magnification: ×12,500. **B,** The cell walls appear to be degraded at intervals (T) to the extent that the wall is digested all the way through. Magnification: ×9,000. **C,** Endocytosis of the cell wall results in digestion vesicles (DV) being formed and carried into the cytoplasm, which tends to be rich in endoplasmic reticulum (ER). Magnification: ×33,500.

results indicate that strain JS314 does not produce the *O*-antigen-containing LPS I.

Glycosyl composition analysis confirmed that the LPS from mutant JS314 was incomplete (Table 3). It lacks 2,3-di-*O*-methylrhamnose, 3-*O*-methylrhamnose, fucose, and quinovosamine. These glycosyl residues, among others, are present in the LPS from the parent 61A101c, and have been reported in the LPSs from other *B. japonicum* strains (Carrion *et al.* 1990; Puvanesarajah *et al.* 1987). The mutant LPS contains 4-*O*-methylmannose, mannose, and glucose in a 1:3:1 ratio. These glycosyl residues, together with 2,3-diamino-2,3-dideoxyhexose and 2-keto-3-deoxyoctonate (KDO), are the only ones found in the mutant LPS. 2,3-Diamino-2,3-dideoxyglucose is the only glycosyl component of the lipid A of *B. japonicum* strains (Carrion *et al.* 1990; Mayer *et al.* 1989). It has also been reported to be a component of the polysaccharide portion of *B. japonicum* 61A123 LPS (Carrion *et al.* 1990). The composition of the LPS from strain JS314 is very similar to the previously described Nod$^-$ mutant, HS123, which lacks the *O*-chain of the LPS (Puvanesarajah *et al.* 1987), and to the isolated LPS II from strain 61A123 (Carrion *et al.* 1990).

The fatty acyl components of the LPSs consist of 3-OH-12:O, 3-OH-14:O, 16:0, 18:1, 24:0, and 27-OH-28:0. There were some minor quantitative differences in these fatty acyl components (i.e., the parental LPS contains larger amounts of 16:0 and 18:1 and a lesser amount of 27-OH-28:0) when compared with the mutant LPS.

DISCUSSION

Kijne (in press) recently pointed out that, in contrast to EPS, intact LPS (LPS I) appears to be required for the formation of stable infection threads in determinate nodules. He suggested that this requirement may reflect a fundamental difference in the way narrow or broad infection threads are formed. One characteristic of narrow infection threads is that the bacteria are in direct contact with the host plasma membrane, in contrast to the situation in broad threads. We have previously documented the close interaction of the bacterial outer membrane with the host plasma membrane at the point of bacterial release from the infection thread (Roth and Stacey 1989a). Kijne (in press) speculated that components of the LPS may be important signals for the endocytotic uptake of single rhizobial cells during the initial formation of narrow infection threads, such as those found in soybean.

Although not conclusive, the results presented here support the notion that an intact LPS I is required for soybean infection and the formation of infection threads. Moreover, mutant JS314 indicates that the induction of plant cortical cell division can be uncoupled from bacterial

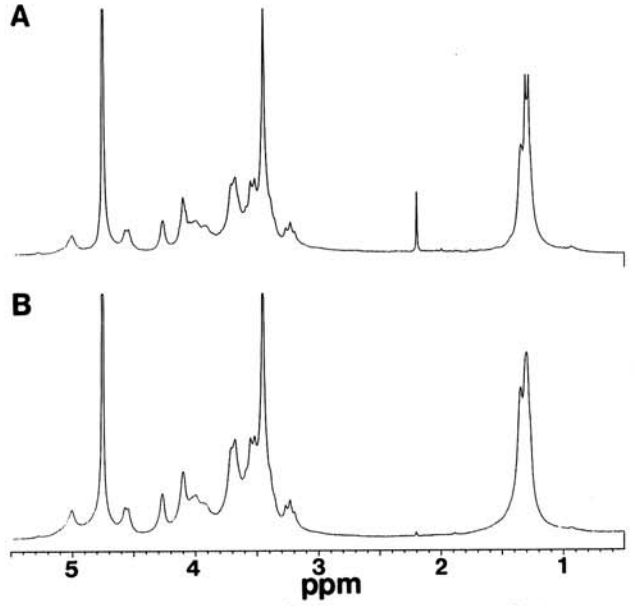

Fig. 6. Proton nuclear magnetic resonance spectra of the exopolysaccharides from *Bradyrhizobium japonicum* strain 61A101c (top) and JS314 (bottom). The spectra were measured on a Bruker AM250 instrument at 300° K.

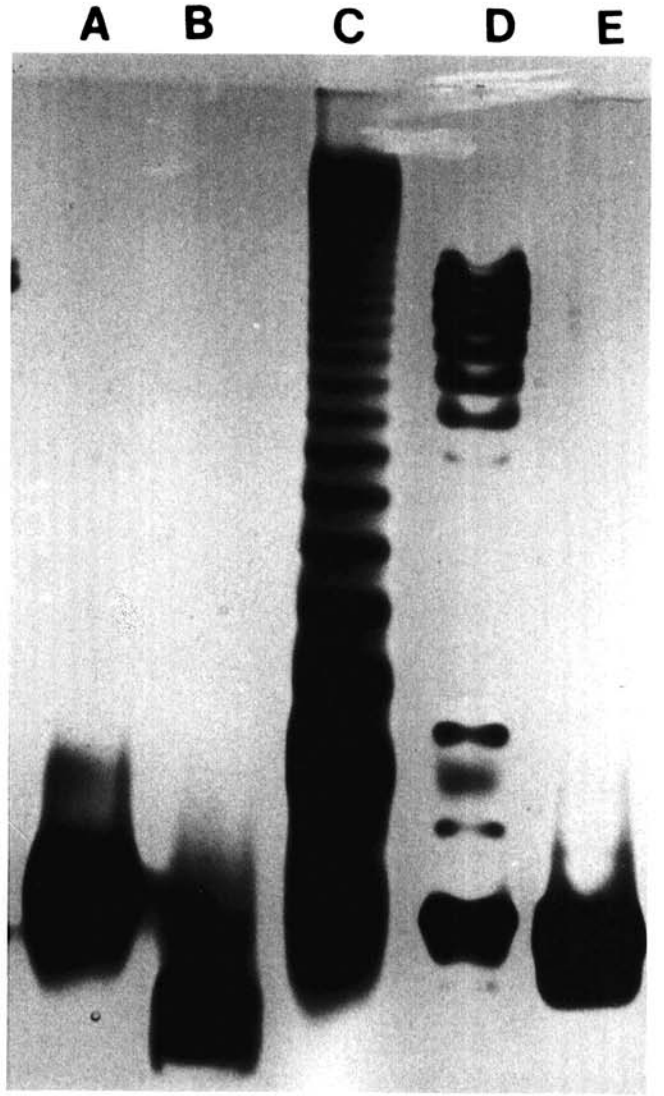

Fig. 7. PAGE analysis of *Bradyrhizobium japonicum* 61A101c (well D) and JS314 (well E) lipopolysaccharides (LPS) in the presence of deoxycholate. Wells A–C contain LPS from *Salmonella typhimurium* R_a, R_d, and wild-type strains, respectively. Bands were visualized by silver-staining. The multiple bands of lower mobility in well D are termed LPSI, and bands of higher mobility in wells D and E are termed LPSII.

infection in soybean. This conclusion is similar to that reached earlier with mutants defective in EPS synthesis in systems in which broad infection threads are formed (e.g., Finan *et al.* 1985). Clearly, the implication is that EPS and LPS play different roles in the formation of indeterminate as opposed to determinate nodules.

This study and our previous work with mutant strain HS123 (Puvanesarajah *et al.* 1987), which also lacks LPS I, documents the importance of LPS to nodulation. Moreover, this work is also instructive with regard to the general structure of *B. japonicum* LPS. Strains 61A101c, 61A123, and USDA110 represent three distinct *B. japonicum* serogroups. Thus far, *B. japonicum* strains all possess 2,3-diamino-2,3-dideoxyglucose in their lipid A. Additionally, LPS II from strains of these three serogroups have similar sugar compositions (Carrion *et al.* 1990; Puvanesarajah *et al.* 1987). These results suggest that all *B. japonicum* strains may possess a common lipid A-core structure. This appears also to be the case for *R. leguminosarum* strains; that is, the lipid core composition of *R. l.* bv. *trifolii* appears to be the same as that from a *R. l.* bv. *phaseoli* or *R. l.* bv. *viciae* LPS (Carlson 1984; Brink *et al.* 1990; Carlson *et al.* 1988; Hollingsworth *et al.* 1989; Carlson *et al.* 1990).

The morphology of the popcorn nodules produced by inoculation of JS314 onto certain cultivars of soybean is very intriguing. The results in Table 2 clearly show that the host response to JS314 differs depending on cultivar and may be genetically controlled. The apparent cell lysis and cell wall degradation occurring at the surface of these nodules might be caused by a plant defensive response to JS314. Djordjevic *et al.* (1988) previously documented a defensive response by the host plant siratro to a mutant strain of *Rhizobium* strain NGR234. This mutant was an adenine auxotroph but also exhibited an overexpression of EPS concomittant with reduced levels of cyclic β-1,2-glucan. The plant response to this mutant included some cortical cell division; however, no infection threads were formed, and there was localized death of epidermal cells at the point of inoculation. These latter effects resemble those seen with the LPS mutant JS314.

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