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Role of Lectins in the Specific Recognition of *Rhizobium* by *Lotononis bainesii*[1]

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ABSTRACT

Fluorescein isothiocyanate (FITC)-labeled lectin purified from the root of *Lotononis bainesii* Baker was bound by cells of five out of seven *L. bainesii*-nodulating strains of *Rhizobium* under culture conditions. With the exception of a strain of *Rhizobium leguminosarum*, strains of noninfective rhizobia failed to bind the root lectin under these conditions. The two nonlectin binding *L. bainesii*-specific strains did not bind root lectin on the *L. bainesii* rhizoplane although this was observed with three other *L. bainesii*-nodulating strains. A single *Rhizobium japonicum* strain bound root lectin on the *L. bainesii* rhizoplane. There was no evidence of an interaction between the *L. bainesii* seed lectin and the *Rhizobium* strains tested.

Root lectin-specific FITC-labeled antibodies were bound to the tips of developing root hairs and lateral growth points of more mature root hairs of *L. bainesii* seedlings. The damaged edges of severed root hairs always bound FITC-labeled root lectin antibody. Seed lectin-specific FITC-labeled antibodies were not bound to the roots of *L. bainesii*. The preemergent root hair region of *L. bainesii* was most susceptible to infection by rhizobia but nodules also emerged in the developing and mature root hair regions. Lectin exposed at growth points on *L. bainesii* root hairs may provide a favorable site for host plant recognition of infective strains of *Rhizobium*.

Legumes may apparently recognize infective species of the nitrogen-fixing bacterium, *Rhizobium*, by a specific reaction between lectin on the host plant root surface and rhizobial cell-surface polysaccharides (5). There is mounting evidence that lectins from certain legumes are bound by cells of *Rhizobium* strains that infect these plants (3, 5, 10), although other reports have been unable to support such findings (*e.g.* 16, 20).

The location of lectins on the legume root, able to react with infective strains of *Rhizobium*, is an important prerequisite for substantiation of the lectin recognition hypothesis. Several authors have examined the interaction between rhizobia and legume root lectins. Lectins isolated from pea root slime (15), white clover roots (10), and root exudate (9) were bound by the species of *Rhizobium* that nodulate these plants. The pea and clover root lectins had properties similar to those of their respective seed lectins. Soybean seedling roots also yielded a lectin with properties characteristic of the soybean seed lectin (11) while immunolatex beads, prepared with seed lectin antibody, were bound by soybean roots (24). There is indirect evidence that the soybean-specific species *Rhizobium japonicum* reacts with lectin on the root of this plant; cells (24) and capsular polysaccharides (13) of *R. japonicum* were bound by soybean seedling roots. This reaction was specifically inhibited by sugar haptens of the soybean seed lectin.

The symbiosis between the perennial legume *Lotononis bainesii* and a red-pigmented species of *Rhizobium* is highly specific (19). We had previously attempted, without success, to demonstrate a reaction between crude lectin preparations from the seed and root of *L. bainesii* and rhizobia specific for this plant (16). Later reports that lectin binding by rhizobia might depend both on culture age (3) and growth media constitution (1), in conjunction with the fact that root lectin of *L. bainesii* was now available in purified form (17) necessitated the re-evaluation of our earlier observations. In this paper, we describe the reaction of rhizobia with seed and root lectins from *L. bainesii*.

MATERIALS AND METHODS

Origin of Bacterial Strains and Plant Material. Strains of *Rhizobium* were from the South African *Rhizobium* Collection, Plant Protection Research Institute, Pretoria. An exception was *R. japonicum* strain I-110 ARS supplied by Dr. L. D. Kuykendall, United States Department of Agriculture, Beltsville, MD. Plants and seeds of *Lotononis bainesii* Baker, used as sources of lectin and for nodulation tests, were from genetically heterogeneous sources in the field.

Media. Cultures were maintained on YEM[2] agar slants (25). In certain tests, mannitol was replaced by an equivalent weight of sodium gluconate (YENG). The defined medium was that of Sherwood (23) but contained 10 mM K-phosphate buffered at pH 7.0. Mannitol was sometimes replaced by sodium gluconate or filter-sterilized arabinose at the same concentration (w/v) in the defined medium. Solid Fahraeus seedling growth medium (25) contained 1.2% (w/v) agar (Oxoid No. 3).

Preparation of FITC-Labeled Proteins. Crude (16) and purified (17) root and seed lectin from *L. bainesii*, as well as lectin-specific antibody (17) was labeled with FITC (16). The purified seed lectin preparation was, however, poorly labeled and could not be used.

Growth and Inoculation of *L. bainesii* Seedlings. Seed was scarified and surface sterilized by 3-min immersion in ethanol followed by 5 min in commercial NaOCl (Jik) containing a drop of Tween 20. The well rinsed seeds were allowed to imbibe water for 2 h before placing swollen seeds on YEM agar plates. The plates were inverted and incubated in the dark at 27°C. Seeds germinated over a 3- to 5-d period. Ten germinated seedlings were placed on seedling agar in an 850-mm-diameter plastic Petri

[1] Results of part of a Ph.D thesis submitted by I. J. L. to the University of Stellenbosch.

[2] Abbreviations: YEM, yeast extract-mannitol medium; YENG, yeast extract-sodium gluconate medium; FITC, fluorescein isothiocyanate; RT, root tip; NRH, no root hair; DRH, developing root hair; MRH, mature root hair; PBS, 150 mM NaCl in 10 mM phosphate buffer (pH 7.0); EPS, extracellular polysaccharide; LPS, lipopolysaccharide; SERH, smallest emergent root hair.

dish (21). Each dish was sealed with parafilm and if necessary, stored at 4°C until day 5. The seedlings were then incubated in an upright position for 2 d in a glasshouse with a 16-h day temperature of 28°C and a night temperature of 23°C. Cultures of *L. bainesii*-specific rhizobia were grown to midexponential phase (A_{620} approximately 0.6) in defined medium containing sodium gluconate. After washing in sterile water, cells were resuspended in water and standardized at 1×10^8 cells/ml. Each seedling was inoculated with 20 μl cell suspension so that the entire length of the rot was moistened. After 30 min in a horizontal position, the Petri dishes were resealed and returned to the glasshouse. To study the pattern of nodulation along the *L. bainesii* root, the position of the RT, NRH, DRH, and MRH zones (4) were marked on the lower Petri dish surface at inoculation. Nodule emergence in the different zones was noted at 24-h intervals. The distance of each nodule from the RT was measured to the nearest 0.5 mm.

Binding Studies with FITC-Labeled Protein. Smears of *Rhizobium* from 7-d-old slant cultures were stained with FITC-labeled lectin preparations as described previously (16) except that smears were mounted in PBS for observation by fluorescence microscopy. Some tests employed cells grown in defined or YENG liquid medium. Slant cultures on the desired medium were used to inoculate liquid medium at an initial concentration of 1×10^6 cells/ml. The fresh cultures were inoculated and sampled at 24-h intervals for 4 to 6 d. Cells were pelleted by centrifugation for 10 min at 17,000*g*, washed with PBS, and resuspended at a concentration of 2×10^6 cells/ml. To 100 μl cell suspension was added 100 μl FITC-labeled lectin (4 to 6 mg/ml). After 15 min, 10 ml PBS was added. The cells were pelleted by centrifugation and resuspended in 100 μl PBS before mounting on a slide. Binding of seed lectin by cells was also tested by pre-incubation of rhizobial cells with 1 ml unlabeled crude seed lectin. After washing, the cells were treated a further 15 min with 100 μl FITC-labeled seed lectin antibody (2 mg/ml) before preparation for observation under the fluorescence microscope.

To determine whether cell fluorescence resulted from specific or nonspecific binding of labeled lectin, the respective seed and root lectin haptens, galactose and lactose, were tested for their ability to remove fluorescent label from the cell surface. A 100 mM solution of the appropriate sugar in PBS was carefully introduced under the coverslip. The nonhapten sugar, glucose, was similarly added to cells as a control.

Lectin binding by rhizobia on the rhizoplane of *L. bainesii* was observed over a 7-d period after inoculation of seedlings. Petri dishes were flooded with PBS containing 0.01% Tween 20. The detergent minimized the entrapment of air bubbles by root hairs and had no perceptible effect on rhizobial adherance or lectin binding. At least two seedlings were carefully removed from the agar surface, briefly rinsed in fresh PBS (no Tween 20), and immersed for 30 min in vials containing 200 μl of the desired FITC-labeled protein. Crude lectins were used at a concentration of 6 to 10 mg/ml. Pure root lectin was at a concentration of 2 mg/ml as was the concentration of FITC-labeled seed or root lectin antibody. After two successive 30-s washes in PBS, the seedlings were mounted in PBS between two plastic strips 0.5 mm thick and set 10 mm apart on a glass slide. The strips were bonded to the slide by epoxy glue and supported a 22 × 40 mm coverslip. This arrangement permitted convenient microscopic observation of the root surface. There was little autofluorescence from plant tissue when observed under a Zeiss UV microscope fitted with a BG12 exciter filter in combination with a No. 53 barrier filter. To test for binding of seed lectin by rhizoplane bacteria, the seedlings were immersed in 10 ml crude seed lectin for 45 min before washing and transfer to FITC-labeled seed lectin antibody for treatment as above. A similar procedure was used with the crude root lectin.

Table I. *Reaction of L. bainesii Root Lectin with L. bainesii-Specific Strains of Rhizobium under Different Culture Conditions*

Rhizobium Strain	*Binding of Lectin to Cells Grown on*[a]			*L. bainesii* rhizoplane
	Defined[b] *medium*	*Yeast extract-mannitol*	*Yeast extract-sodium gluconate*	
XCT5	−	−	+	nd[c]
XCT8	−	−	+	+
XCT10	−	−	+	nd
XCT12	−[d]	−	+	+
XCT14	−	−	−	−
XCT16	−[d]	−	−	−
XCT17	−[d]	−	+	+

[a] +, Specific binding of FITC-labeled root lectin to cell surface; −, negative reaction.

[b] Defined medium; sodium gluconate was the carbon source, there was no yeast extract.

[c] nd, not determined.

[d] Also cultured in defined medium supplemented with arabinose or mannitol.

Extraction of Rhizobial Polysaccharides. One-week-old cultures of *L. bainesii*-specific rhizobia were washed from YENG agar and homogenized for 2.5 min in 150 mM NaCl (saline) using a Sorvall Omni-Mixer set at full speed. Each cell homogenate was clarified by centrifugation for 60 min at 32,800*g*. In one extraction procedure, EPS (fraction A) was precipitated from the supernatant with cetylpyridinium chloride (22). After removal of the precipitate by centrifugation (10 min at 6,000*g*), a remaining polysaccharide fraction (fraction N) was precipitated by the addition of cold acetone:ethanol (1:1, v/v). A subsequent extraction procedure used a different set of 7-d-old *Rhizobium* cultures. After centrifugation of the homogenized cell suspension, the supernatant was decanted. A viscous jelly-like layer (fraction V) above the cell pellet was carefully scraped off and stored. The supernatant liquid was exhaustively dialyzed against distilled H_2O and soluble polysaccharide (Fraction S) precipitated by the addition of cold acetone-ethanol. With the exception of fraction V, all EPS preparations were freeze dried after dialysis against distilled H_2O. Cell wall LPS was extracted (26) from 7-d-old cultures of *L. bainesii* specific rhizobia after the cells had been washed twice with saline and freeze dried.

Reaction of Root Lectin with Polysaccharides. Purified root lectin (1 mg/ml) was tested for a possible reaction with LPS (1 mg/ml) using the hemagglutination inhibition and agar gel double diffusion tests previously described (17). Solutions of the different EPS preparations in PBS were generally highly viscous and not suitable for hemagglutination inhibition tests. Instead, loopsful of EPS fractions A and N, dissolved in water, were spread on slides, air dried, and reacted with FITC-labeled purified root lectin (2 mg/ml) as described for cell smears (16). After a 15-min rinse in PBS, the EPS films were observed for fluorescence. In a further test, solutions of the EPS fractions S (1 mg/ml) and V (unknown concentrations) were also added to wells in agar double diffusion gels (17). Root lectin (1 mg/ml) was added to the center well after the liquid in the polysaccharide containing wells had been absorbed into the agar.

RESULTS

Reaction of *L. bainesii* Lectins with cells of *Rhizobium*. Strains of the various *Rhizobium* spp. tested were grown in solid- and liquid-defined and yeast extract-containing media in attempts to induce the synthesis of *L. bainesii* lectin receptors. Three strains of *L. bainesii*-specific rhizobia were cultured in defined medium containing either mannitol, arabinose, or sodium gluconate (Table I). Whereas the exponential growth rate of the strains was

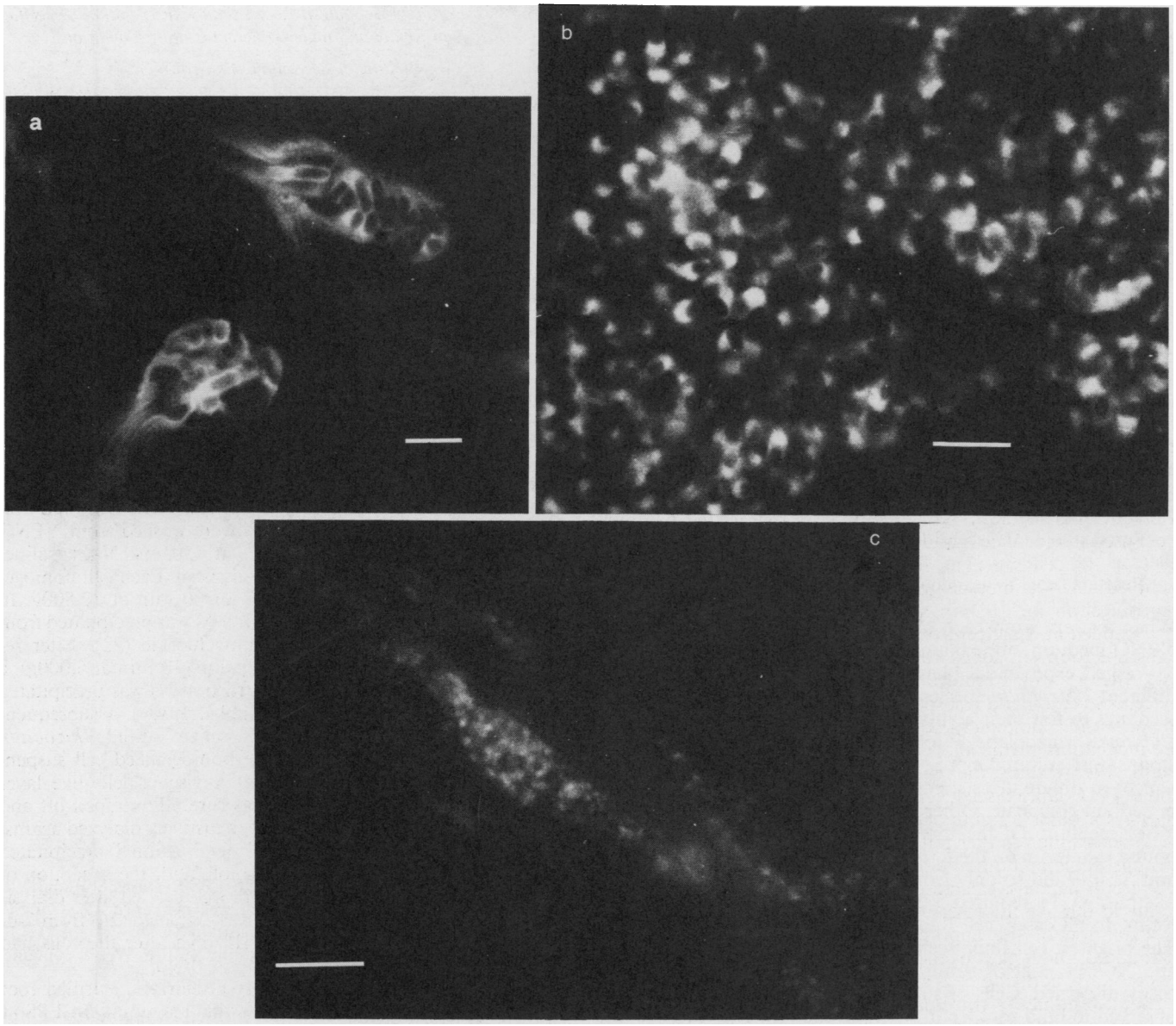

FIG. 1. FITC-labeled *L. bainesii* root lectin bound by *L. bainesii*-nodulating strains XCT12(1a), and XCT8(1b) grown on YENG medium, and strain XCT12(1c) on *L. bainesii* root hairs. Bars equal 5 μm in (a) and (b), but 20 μm in (c).

similar irrespective of the sugar used, sodium gluconate prolonged growth of strains XCT12 and XCT17, but not strain XCT16. Mannitol was used for the other *Rhizobium* spp. grown in this medium.

The reaction of root lectin with strains of *L. bainesii*-specific rhizobia is summarized in Table I. None of the strains bound FITC-labeled root lectin at any stage of their growth in defined medium containing either arabinose, mannitol or sodium gluconate, or when cultured in YEM medium. However, substitution of sodium gluconate for mannitol in YEM agar medium elicited root lectin receptor production by five of seven strains of *L. bainesii*-specific rhizobia (Table I). The reaction was hapten reversible; lactose but not glucose specifically removed lectin from fluorescent cells. Strains XCT5, XCT10, XCT12, and XCT17 were similar in their production of copious lectin-binding material (Fig. 1a). Strain XCT8 formed less slime in YENG medium and had a granular appearance when cultured on agar. This strain produced less abundant root lectin receptor material at the poles of individual cells (Fig. 1b). Of five strains of *L. bainesii*-specific rhizobia cultured for 4 d in YENG liquid medium, strains XCT14 and XCT16 failed to bind root lectin at any stage of growth (Table I); strains XCT12 and XCT17 bound lectin from 24 h onwards. Hapten-reversible fluorescence was associated with large irregular globules or stringy material not always associated with cells. This made it impossible to follow the course of receptor production by individual cells of these strains. The intensity of fluorescence increased as the quantity of the amorphous receptor material increased. Cells of strain XCT8 were observed to fluoresce only at 24 h and on the 4th d. Lectin receptor material was located on cells of this strain as in Figure 1b. Cells of strain XCT8 always aggregated in liquid medium; thus, it was not possible to estimate the proportion of fluorescent cells present at any stage of growth.

Three strains of each of the species *R. leguminosarum*, *R. trifolii*, *R. phaseoli*, *R. meliloti*, *R. japonicum*, and *R. lupini* failed to bind root lectin during growth in defined medium. When cultures were grown in YEM medium, only one strain of *R. leguminosarum*, bound lectin in a hapten-reversible manner.

Table II. *Reaction of L. bainesii Root Lectin with Cell Surface Polysaccharides from L. bainesii-Specific Strains of Rhizobium*

Rhizobium Strain	LPS[b]	Binding of Root Lectin by[a]			
		Fraction A[c]	Fraction N[c]	Fraction S[d]	Fraction V[d]
XCT5	−	+	+	+	+
XCT8	−	+	+	+	+
XCT10	nd	nd	nd	+	+
XCT12	−	+	+	+	+
XCT14	−	+	−	−	−
XCT16	−	−	−	−	−
XCT17	−	−	+	+	+

[a] EPS fraction A, cetylpyridinium chloride-precipitated polysaccharide; EPS fraction N, residue precipitated with acetone-ethanol after removal of cetylpyridinium chloride-precipitated EPS material; EPS fraction S, soluble polysaccharide precipitated with acetone-ethanol; EPS fraction V, viscous polysaccharide sedimented from cells by centrifugation; +, positive reaction of polysaccharide with lectin; −, no reaction of polysaccharide with lectin; nd, reaction not determined.

[b] Reaction tested by hemagglutination inhibition and agar double diffusion methods.

[c] Reaction of FITC-labeled lectin with polysaccharide film on glass slides.

[d] Reaction tested by agar double diffusion method.

When a strain from each of the above species was cultured in YENG medium, none bound root lectin.

Washed exponential phase cells of strains from each of the different *Rhizobium* species were used to inoculate *L. bainesii* seedlings to test their ability to bind lectin when grown on the *L. bainesii* rhizoplane. Prior to inoculation, *L. bainesii*-specific strains were cultured in YENG medium and in defined medium which, in different tests, contained either arabinose, mannitol, or sodium gluconate. Other species of rhizobia were cultured in YEM medium. Of the three *L. bainesii*-specific strains which bound root lectin on the *L. bainesii* rhizoplane (Table I), XCT12 and XCT17 did so only from the 3rd d after inoculation. Cells of strain XCT8 required 5 d before they were observed to bind lectin. In all cases, cell fluorescence was quenched by lactose. The brightest cell fluorescence was by strains XCT12 (Fig. 1c) and XCT17; that of strain XCT8 was more discrete and less easily discerned. Cells of strains XCT14 and XCT16 were not observed to bind root lectin when on the *L. bainesii* rhizoplane (Table I). The use of different media to culture cells prior to inoculation had no influence on their lectin-binding ability.

Of seven strains representing other *Rhizobium* spp., only a strain of *R. japonicum* (I-110 ARS) bound root lectin on the rhizoplane. Cells of this strain bound lectin for 48 h after inoculation, then abruptly lost their ability. It did not bind lectin when cultured on YEM medium. A second *R. japonicum* strain tested, did not bind lectin on the *L. bainesii* rhizoplane over the 7-d test period.

Similar results were obtained when FITC-labeled root lectin antibody was used to detect root lectin binding by cells of the above rhizobia incubated in the crude root lectin solution. On the other hand, the different seed lectin preparations did not react in a specific manner with rhizobia nor were labeled seed lectin-specific antibodies bound by cells after pretreatment with seed lectin. Various cell preparations, particularly those of fast growing strains grown on YEM, exhibited some nonspecific adsorption of FITC-labeled preparations of the root and seed lectins.

Reaction of Root Lectin with Cell Surface Polysaccharides from *L. bainesii*-specific *Rhizobia*. The cell wall LPS and capsular EPS of *L. bainesii*-specific rhizobia were extracted and tested for their reaction with the *L. bainesii* root lectin. Double diffusion precipitin bands were not formed by the highly soluble LPS preparations against root lectin, nor were they able to inhibit root lectin hemagglutination (Table II).

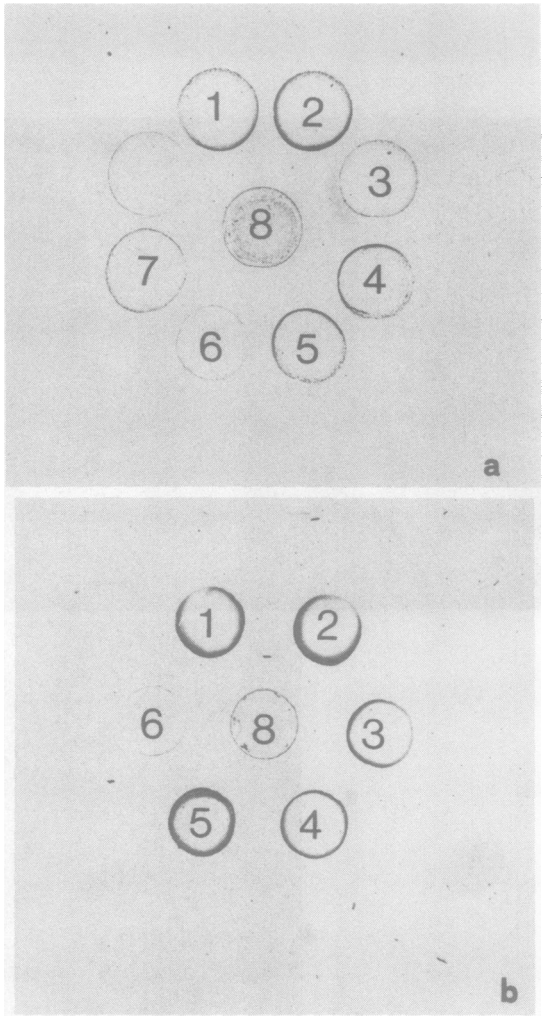

FIG. 2. Interaction of root lectin with EPS fractions S(a) and V(b) from *L. bainesii*-specific rhizobia. Each well contained 15 μl of EPS from: 1, strain XCT17; 2, strain XCT5; 3, strain XCT8; 4, strain XCT10; 5, strain XCT12; 6, strain XCT14; 7, strain XCT16. Well 8 contained 15 μl root lectin solution.

Smears of the EPS fractions A and N from some strains, cells of which bound root lectin, were visible as fluorescent spots after reaction with FITC-labeled root lectin (Table II). An exception was the EPS fraction A from strain XCT17 which did not bind lectin (Table II). In addition, the EPS fraction A from strain XCT14 bound root lectin (Table II). Polysaccharides that did not bind lectin were visible as opaque films under the phase-contrast microscope. Labeled root lectin that was bound by the different EPS preparations could be removed by PBS containing 100 mM lactose. Glucose was ineffective in this respect.

Attempts were also made to demonstrate an interaction between root lectin and the different EPS fractions S and V, by agar gel double diffusion. After 2 d incubation, no precipitin bands had developed except for a diffuse band against the EPS fraction S of strain XCT8 (Fig. 2a). Translucent polysaccharide was, however, observed at the base of all the other EPS-containing wells. When the washed and dried agar was subsequently stained for protein, only wells containing EPS from strains whose cells had previously been observed to bind root lectin, were stained (Table II, Fig. 2). The EPS fraction V from strain XCT16 was on a separate plate and is not included in Figure 2b. Neither

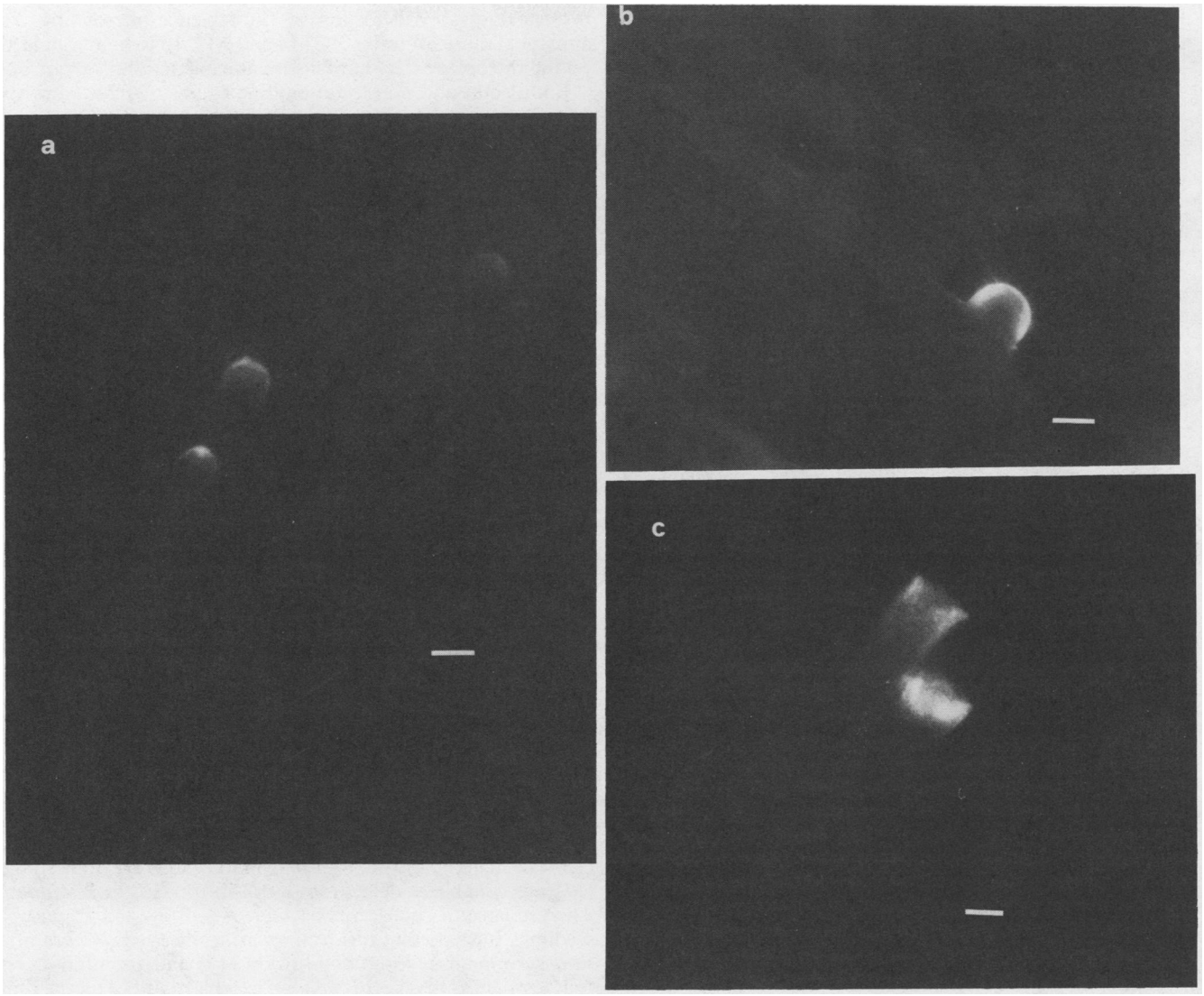

FIG. 3. FITC-labeled root lectin antibody bound to *L. bainesii* root hairs. a, Root hairs in DRH zone; b, root hair in MRH zone; c, severed root hairs in MRH zone. Bar equals 10 μm.

EPS fraction, S nor V from strains XCT14 and XCT16, was stained (Table II, Fig. 2).

Location of Lectin on the Root Surface of *L. bainesii*. Root lectin-specific FITC-labeled antibody was bound by the tips of root hairs in the DRH zone situated close to the root tip of 3- to 10-d-old *L. bainesii* seedlings (Fig. 3a). Short lateral growth points of more mature root hairs also bound root lectin antibody (Fig. 3b). Severed edges of root hairs that were broken when seedlings were removed from agar plates always bound the root lectin antiserum (Fig. 3c). There was no evidence that FITC-labeled seed lectin-specific antibody was bound to the surface of 3- to 10-d-old *L. bainesii* seedlings.

Pattern of Nodulation on *L. bainesii* Roots. *L. bainesii* seedlings, grown on Fahraeus seedling agar for 3 d, were inoculated with midexponential phase cultures of strain XCT16. The sequence of appearance of nodules along the root was noted over a 6-d period after inoculation. Most nodules developed in the NRH zone but a few emerged in the DRH and MRH zones (Fig. 4). The first nodules were noted on day 3. The bulk of the nodules in the NRH zone emerged on day 5 (Fig. 4). The sequence of appearance of nodules in the other zones was less well defined (Fig. 4). The profile of nodule development relative to the length of the root shows that most nodules developed in a sharply defined zone below the SERH mark which was about 1.5 mm above the RT mark (Fig. 5). Few nodules developed on the region of the root immediately below the RT mark (Fig. 5).

DISCUSSION

Most studies of lectin binding by rhizobia used lectins isolated from legume seeds. *L. bainesii* was novel in containing a root lectin not present in the seed of this plant (17) that reacted with certain strains of *Rhizobium* specific for *L. bainesii*. This result was consistent with the lectin recognition hypothesis (5). There was, however, no evidence of an interaction between the seed lectin of *L. bainesii* and *Rhizobium*.

Culture conditions were important for the production of root lectin receptors on the cell surface of *L. bainesii*-specific rhizobia. Cells did not bind the lectin when grown in a defined medium containing different sugars or in YEM medium. Similar observations with strains cultured on YEM medium have been recorded (16). Substitution of sodium gluconate for mannitol in YEM medium stimulated lectin receptor production by five of seven *L. bainesii*-specific rhizobia. The reason for this is not known but may be related to the heavier growth of the lectin-

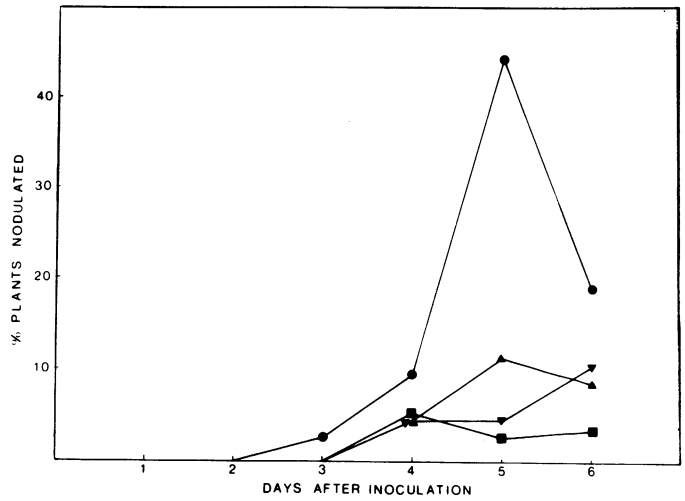

FIG. 4. Sequence of nodulation in different zones of root hair development of *L. bainesii* seedlings (100 plants) grown on Fahraeus agar in Petri dishes. ●, NRH zone; ▲, DRH zone; ■, MRH zone; ▼, zone of root hairs below RT mark.

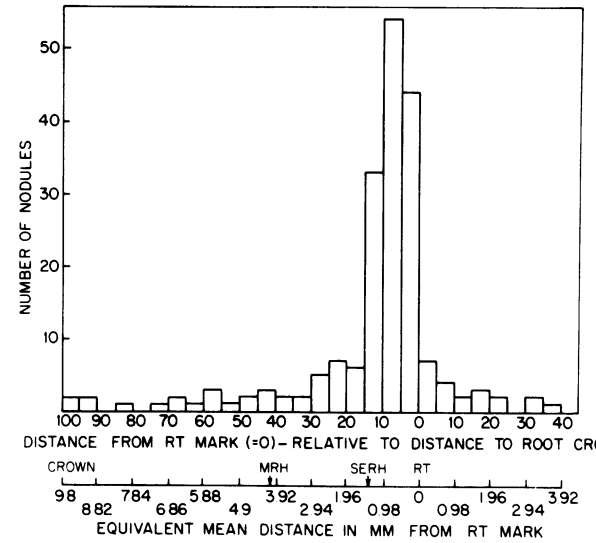

FIG. 5. Profile of nodulation along the root of *L. bainesii* seedlings grown on Fahraeus agar medium. The distance of each nodule from the RT mark was calculated relative to the distance from the RT mark to the root crown of the plant. The mean distance from the RT mark to the root crown of 115 plants was 9.8 ± 3.3 mm. The approximate positions of the SERH and the lowest MRH on the root are indicated. Out of 115 plants, 100 developed nodules.

binding strains XCT12 and XCT17 when sodium gluconate was substituted for other sugars in the defined medium. Growth of the non-lectin-binding strain XCT16 was not similarly affected. Yeast extract nutrients in the YENG medium were also important since lectin was not bound by strains cultured in defined medium when this contained sodium gluconate.

Cell surface lectin receptors may be induced on rhizobia cultured in association with the host root (1, 18). While three *L. bainesii*-specific strains that bound root lectin in YENG medium also did so on the *L. bainesii* rhizoplane, two non-lectin-binding strains in YENG medium, failed to bind lectin on the rhizoplane. The production of lectin receptors by strains on the *L. bainesii* rhizoplane appeared less abundant than in culture and was only observed when nodulation had already commenced. This was well after the initial lectin-*Rhizobium* recognition event ought to have occurred. The earlier production of receptor material may thus have escaped detection by fluorescence microscopy. The apparent failure of strains XCT14 and XCT16 to bind root lectin on the rhizoplane may have a similar explanation.

Both lectin and non-lectin-binding strains of *L. bainesii*-specific rhizobia synthesized EPS when grown on YENG medium. This had previously not been observed with a *L. bainesii*-specific strain cultured in liquid medium containing yeast extract and 5% mannitol (7). The reaction of root lectin with EPS fractions (Table II) from the *L. bainesii*-specific strains was similar to that obtained with cells of these strains (Table I). The observation that the apparently acidic EPS fraction A from strain XCT14 did bind root lectin suggested that strain XCT14 may synthesize lectin receptor material, although cells and the EPS fractions S and V of strain XCT14 did not react with the lectin. This discrepancy, together with differences in lectin binding by EPS fractions A and N from strain XCT17, requires clarification. There was no evidence of an interaction between the root lectin and cell wall LPS from *L. bainesii*-specific rhizobia. These strains thus resembled other fast and slow growing rhizobia (6, 8, 14) in the capsular nature of their lectin receptor material. A strain of *R. leguminosarum* grown on YEM medium and of *R. japonicum* on the *L. bainesii* rhizoplane also bound root lectin. This supported an earlier finding (1) that noninfective strains may bind legume lectin and that the induction of rhizobial lectin receptors by plant factors is not necessarily host-specific.

The location of lectin on *L. bainesii* root hairs resembled that of white clover (10) and pea (12). FITC-labeled root lectin antibody was bound to the tips of developing root hairs as well as lateral growth points on more mature root hairs of these seedlings (Fig. 3, a and b). Root hairs that had been accidentally severed also bound root lectin at the broken edges regardless of their location along the root (Fig. 3c). These observations suggest that the *L. bainesii* root lectin was only briefly exposed at primary and secondary growth points on the root hair. The lectin may then have been incorporated within, or overlaid by, additional cell wall material during root hair maturation. Previous studies (17) were unable to detect root lectin in the seed of *L. bainesii*. The presence of root lectin on the roots of 3-d-old *L. bainesii* seedlings suggests that this lectin was synthesized *de novo* after seed germination. Immunodiffusion (17) and immunofluorescent tests failed to detect *L. bainesii* seed lectin in or on the roots of this plant. At present it is not known whether other tissues of *L. bainesii* contain lectins corresponding to seed and root lectins.

In common with several other legumes (2, 4), most nodules emerged in the NRH zone of seedlings of *L. bainesii* inoculated with strain XCT16. This strain was arbitrarily chosen for nodulation studies before its non-lectin-binding character had been established. However, the nodulation patterns of the other *L. bainesii*-specific strains, observed during lectin-binding studies on the rhizoplane of this plant, did not apparently differ from that of strain XCT16. The development of nodules in the DRH and MRH zones appeared similar to that of lucerne (2). Despite the apparent inability of the inoculant strain XCT16 to bind root lectin (Tables I and II), the location of the prime infectible zone near the *L. bainesii* root tip may have been related to the detection of root lectin at the tips of developing seedling root hairs. The lectin recognition hypothesis suggests that root lectin should be present on the root surface at infectible sites where recognition of, and infection by, symbiotic rhizobia occurs. Root lectin was not, however, detected on the epidermal cell surface of the NRH zone, although this was the region most susceptible to infection by rhizobia. A possible explanation might be that there was insufficient root lectin on pre-emergent root hair cells for detection with FITC-labeled antibodies. Nevertheless, root lectin exposed at growth points on the cell wall of developing and more mature root hairs may have provided suitable infection sites for nodulation in the different regions of the *L. bainesii*

root. The nature of the infection process in *L. bainesii* has, however, yet to be described. The relation in rhizobial infection on *L. bainesii* to the distribution of root lectin on the root surface will require careful definition before a role in the symbiotic recognition process can be ascribed to this lectin.

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