Fluorescein Isothiocyanate-Labeled Lectin Analysis of the Surface of the Nitrogen-Fixing Bacterium *Azospirillum brasilense* by Flow Cytometry

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Received 16 December 1987/Accepted 28 April 1988

*Azospirillum brasilense* is a nitrogen-fixing bacterium which grows in close association with the roots of grasses without the formation of nodules. This organism may be found on root mucilages of various agriculturally important plants (18, 35) or may establish infection sites within the root cortex (33, 35). *A. brasilense* has been observed to stimulate plant growth by producing plant growth hormones (33), by enhancing the uptake of nutrients (3, 21), and by increasing the nitrogen content of plants (7). The nature of the association of *A. brasilense* to a host is unresolved; however, it has been suggested by Kreig (20) that bacterial strain specificity exists for *C*. and *C*. grasses, and Okon (26) has reported that there may be specific sites for bacterial colonization on roots.

The role of bacterial cell surface recognition in the colonization of host tissue has been well documented for animal (31) and plant (11) systems. Analysis of the cell surface by using lectins has yielded useful information for the characterization of sugars in bacteria capsule and cell walls (5, 9, 28, 32). Lectin-cell interactions may be detected by agglutination; however, quantitation of the degree of agglutination is not easily achieved. Flow cytometry, a highly analytical technique, has been used to evaluate cell populations (4, 30); however, this application has been almost exclusively with eucaryotic cells.

In this study, we evaluated the binding of fluorescence-labeled lectins to the cell surface of *A. brasilense* by flow cytometry. The results were compared with those of agglutination studies in order to evaluate the usefulness of flow cytometry for the study of lectin-cell interactions.

**MATERIALS AND METHODS**

*Cultivation of bacteria.* *A. brasilense* ATCC 29145 was grown in the malate-salt medium described by Albrecht and Okon (1). Half of the cultures received NH₄Cl (0.1%) as the nitrogen source, while the remainder contained no added nitrogen salts, with cells fixing atmospheric nitrogen. Where appropriate, 1.5% agar was added to the medium to provide a solid surface for colony development.

Bacteria used for lectin binding were obtained from non-stirred cultures grown at 30°C in 40 ml of medium in a 250-ml Erlemeyer flask. Cells were harvested by centrifugation at 12,000 × g for 10 min at room temperature, washed twice in phosphate-buffered saline (PBS) solution containing 0.85% NaCl and 0.01 M sodium phosphate (pH 7.4). Cell densities were expressed as optical density values at 560 nm, and a direct correlation was found between optical density and CFU.

**FITC-lectin survey.** Cells of *A. brasilense* grown under N₂-fixing or NH₄⁺-assimilating conditions were examined for lectin binding by using the following fluorescein-isothiocyanate (FITC)-labeled lectins (FITC-lectins): *Griffonia simplicifolia II* agglutinin (GS I), also referred to as Bandeiraea simplicifolia I agglutinin (37); *G. simplicifolia II* agglutinin (GS II), also known as *B. simplicifolia II* agglutinin (10); *Lotus tetragonolobus* agglutinin (LTA) (27); *Canavalia ensiformis* agglutinin (Con A) (29); *Triticum vulgaris* agglutinin (WGA) (25); *Glycine max* agglutinin (SBA) (22); and *L. flavidus* agglutinin (LFA). All FITC-lectins were obtained from Sigma Chemical Co. (St. Louis, Mo.), except for LFA which was purchased from E-Y Laboratory (San Mateo, Calif.).

**Binding of FITC-lectins.** Cells of *A. brasilense* were diluted in a solution containing 0.13 M NaCl and 10 mM Tris hydrochloride (pH 7.4). Portions (0.5 ml) of the cell suspension containing 5 × 10⁹ cells were placed in glass centrifuge tubes (13 by 125 mm), and the cells were washed twice in the appropriate buffer for each lectin. The buffer solution for FITC-LFA was 50 mM Tris hydrochloride (pH 7.5) containing 10 mM NaCl and 20 mM CoCl₂. For FITC-Con A, the buffer solution was 5 mM Tris hydrochloride (pH 7.0) with 15 mM NaCl, 1 mM CaCl₂, and 1 mM MnCl₂. For other FITC-lectins (SBA, WGA, LTA, GS I, and GS II), the buffer was PBS (pH 7.45), but for FITC-GS I and FITC-GS II, 5 mM CaCl₂ was added to the buffer. The binding of FITC-
lectins to the washed bacteria was accomplished by the incubation of 5 × 10⁸ cells of A. brasilense with 50 μg of FITC-lectin in 0.5 ml of buffer solution. After 30 min at 25°C, 1 ml of PBS was added to each tube, and the cells were collected by centrifugation at 2,000 × g for 10 min at room temperature. The lectin-treated cells were washed twice in PBS solution before being fixed in PBS solution containing 1% paraformaldehyde. Tubes containing fixed cells were capped and stored in the dark at 4°C for less than 36 h before examination by flow cytometry.

The inhibition of lectin binding was examined by the addition of sugars, known to be appropriate lectin receptors, to cell suspensions of A. brasilense. Sugars were added at a 0.1 mM final concentration to the buffers used to wash cells and to the FITC-lectin solutions. The inhibition of lectin binding by sugar was determined by comparing bound FITC-lectin with bacterial cells in the absence of added sugars.

Flow cytometry analysis. Bacteria labeled with FITC-lectins were examined with a FACS II cytometer (Becton Dickinson and Co., Paramus, N.J.) equipped with a 37-μm (pore size) filter, a 75-μm (pore size) orifice, and a 0.3 neutral density filter. The photomultiplier tube was set at 500 V, and a variable gain of 4 was used for all runs. The calibration and standardization of the flow cytometer were in accordance with manufacturer specifications. Suggestions in published procedures were also followed (13, 36). Fluorescent microbeads (Flow Cytometry Products Corp., Research Triangle Park, N.C.) were used as standards for fluorescence and volume. A computer (model 9816; Hewlett-Packard Co., Palo Alto, Calif.) was used to process fluorescence and volume data.

Agglutination. Cells of A. brasilense were grown for 24 h under N₂-fixing or NH₄⁺-assimilating conditions and examined for lectin-mediated agglutination according to the published procedure (5). Cells were washed in a buffer solution containing 0.85% NaCl and 10 mM Bis-Tris hydrochloride (pH 7.4). The cells were diluted to a number 4 McFarland barium sulfate standard with the NaCl-Tris buffer, and 0.1 ml of cell suspension was incubated with 100 μg of lectin. The suspension was gently mixed on a slide rocker for 20 min at 22°C, and agglutination was subjectively evaluated by using a microdilution reading mirror. The lectins used in the agglutination study were obtained from E-Y Laboratory and include the lectins used for FITC fluorescence study plus Arachis hypogaea agglutinin (23), Trichosanthes kinlowlíi agglutinin, Ulex europaeus agglutinin (24), Solanum tuberosum agglutinin (2), Phaseolus vulgaris agglutinin (19), Lens culinaris agglutinin (16), and Dolichos biflorus agglutinin (12).

Microscopy. Cells which were exposed to FITC-lectins were examined by epifluorescence with a Leitz-Witzlar microscope equipped with a number 3 dichroic mirror and a K510 yellow filter. The India ink procedure was used to assess capsules in cells grown by NH₄⁺ assimilation or N₂ fixation.

Capsule production by A. brasilense was evaluated by transmission electron microscopy, with a modification of the method of Jones et al. (17). Epon 812 disks were cast in size 3 beam capsule lids and were cleaned in 100% ethanol and sterile water before being placed in Erlenmeyer flasks inoculated with A. brasilense. After stationary incubation for 24 h, the disks were removed from the N₂ or NH₄⁺ cultures and placed in a solution containing 2% aqueous glutaraldehyde, 0.1 M cacodylate buffer (pH 7.3), and 1,500 ppm (1,500 μg/ml) ruthenium red. The fixed bacteria were washed three times in 0.15 M cacodylate buffer and stained with 2.5% osmic acid and 1,500 ppm ruthenium red for 1 h. The disks were washed in cacodylate buffer, dehydrated in ethanol, and exposed to propylene oxide for 30 min before being infiltrated with Epox 812 resin. The disks were then placed in size O beam capsules containing fresh resin and heat polymerized. Thin sections were cut with a diamond knife, and samples were stained with 2% uranyl acetate and 1% lead citrate.

Exopolysaccharide assay. Bacteria were removed from the liquid culture by centrifugation at 12,000 × g for 10 min. The supernatant was passed through a membrane filter (Millipore Corp., Bedford, Mass.) with a pore diameter of 0.45 μm and assayed for hexose by the procedure of Herbert et al. (15), with glucose as the standard.

RESULTS

Capsule demonstration. A. brasilense produced a discrete capsule when grown under either N₂-fixing or NH₄⁺-assimilating conditions. The capsule was readily observed by light microscopy with India ink staining and was apparent at all phases of growth. Bacteria prepared for flow cytometry displayed capsules, indicating that the capsule was not removed by repeated cell manipulations. The presence of capsules was confirmed by transmission electron microscopy of thin sections of A. brasilense. The capsule around NH₄⁺-grown cells is apparent in Fig. 1, and similar capsular morphology was observed with cells fixing N₂.

Examination of lectin bound to A. brasilense by epifluorescence microscopy revealed that FITC-lectins were uniformly distributed on the cell surface. In Fig. 2, the binding of FITC-SBA to both A. brasilense individual cells and cell aggregates is shown. Similarly, with FITC-Con A, FITC-GS I, FITC-GS II, FITC-WGA, FITC-LFA, or FITC-LTA, lectin binding was uniformly distributed on the surface of
individual or aggregated cells. Observation of cells labeled with FITC-lectins, prior to flow cytometry measurements but after repeated manipulations, revealed levels of fluorescence which were comparable with that seen with initial cell suspensions.

**Flow cytometry.** The binding of seven FITC-lectins to cells of *A. brasilense* is shown in Table 1. The greatest binding to cells was observed with FITC-GS II and FITC-GS I, whereas intermediate binding was observed with FITC-WGA, FITC-SBA, and FITC-Con A. The lowest level of binding was with FITC-LFA and FITC-LTA.

The percentage of cells which bound lectin depended on culture age and the nitrogen source in the growth medium. In general, fewer cells from a 45-h culture were found to bind lectin than did cells from a 12-h or 24-h culture. However, more cells from a 45-h culture grown on N2 were found to bind LFA and Con A than did younger cells grown on the same nitrogen source. Cells from 24- and 45-h cultures grown on N2 bound more lectins than did cells of comparable age grown on NH4+, except for LFA binding to 24-h cells. For the 15-h cultures, cells grown on NH4+ bound LFA, Con A, SBA, or GS I to a greater extent than did cells grown on N2. The 24-h culture most closely reflected the log phase for both N2 and NH4+ cultures (Fig. 3).

The reproducibility of the FITC-lectin binding system was examined by using cells derived from eight distinct populations of *A. brasilense* grown under N2-fixing conditions. Triplet tests on the isolates produced an average value of 43.8% for the cells binding FITC-WGA with a range of ±3.3 and a standard error of ±4.7 (Table 2). While the percentage of cells binding FITC-WGA in this study was less than 68% (as reported in Table 1), the concentration of lectin in the previous assay (Table 1) was twice as great as that used in the triplicate tests (Table 2).

The examination of cells which bound FITC-lectins revealed that fluorescence intensity depended on FITC-lectin in the binding assay. The magnitude of fluorescence intensity is graphically presented as the percentage of bacteria detected at various fluorescence intensities (Fig. 4 to 7). With FITC-Con A binding to N2-fixing cells, at a fluorescence level of 6.5 on a linear scale, 64% of the cells exposed to 50

**TABLE 1.** Binding of FITC-lectins to *A. brasilense* as determined by fluorescence-activated flow cytometry.

<table>
<thead>
<tr>
<th>Lectin added (sugar specificity)</th>
<th>Nitrogen source for growth</th>
<th>% of cells of indicated age binding lectin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>12 h 24 h 45 h</td>
</tr>
<tr>
<td>GS II (N-acetyl-β-D-glucosamine and N-acetyl-α-D-glucosamine)</td>
<td>NH4+</td>
<td>88 87 44</td>
</tr>
<tr>
<td>GS I (α-D-galactose &gt; N-acetyl-α-D-galactosamine)</td>
<td>N2</td>
<td>91 95 84</td>
</tr>
<tr>
<td>WGA (N-acetyl-β-D-glucosamine and N-acetyl-α-D-glucosamine)</td>
<td>NH4+</td>
<td>86 86 38</td>
</tr>
<tr>
<td>SBA (N-acetyl-α-D-galactosamine &gt; N-acetyl-β-D-galactosamine)</td>
<td>N2</td>
<td>81 94 82</td>
</tr>
<tr>
<td>Con A (α-D-mannose &gt; α-D-glucose)</td>
<td>NH4+</td>
<td>48 56 35</td>
</tr>
<tr>
<td>LFA (sialic acid)</td>
<td>N2</td>
<td>56 68 43</td>
</tr>
<tr>
<td>LTA (α-L-fucose)</td>
<td>N2</td>
<td>39 42 72</td>
</tr>
</tbody>
</table>

a Data were based on the analysis of 10,000 cells for each measurement. Median values of triplicate experiments are presented, and the range was less than 2% for each triplicate run.

**TABLE 2.** FACS analysis of FITC-wheat germ agglutinin bound to cells of *A. brasilense* grown under nitrogen-fixing conditions.

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>% of cells binding wheat germ agglutinin*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>49</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
</tr>
</tbody>
</table>

a A total of 10,000 cells were counted for each isolate in this FACS experiment. The amount of lectin was 25 µg/0.5 ml of reaction mixture. The mean value (± standard error of the mean) was 43.8 ± 4.7.

b Isolate number 8 was the culture used for the other lectin studies in this report.
Fluorescence

FIG. 4. Fluorescence intensity of FITC-Con A-labeled cells of *A. brasilense*. NH₄⁺-assimilating (A) and N₂-fixing (B) cultures were examined with 10 (○), 30 (△), or 50 (□) μg of FITC-Con A. 

μg of lectin, 34% of the cells exposed to 30 μg of lectin (Fig. 4B), and 6% of the cells exposed to 10 μg of lectin (data not shown) were observed to fluoresce. Also, with FITC-Con A, the maximum level of fluorescence changed with the amount of lectin added to N₂-fixing cells. With NH₄⁺-assimilating bacteria, the percentage of cells at a fluorescence level of 6.5 was less with 30 μg of FITC-Con A than with 10 or 50 μg of FITC-Con A (Fig. 4A). The fluorescence of FITC-SBA binding to cells grown on NH₄⁺ directly depended on lectin concentration at a fluorescence level of 3.5 (Fig. 5), and cells from N₂ culture displayed a fluorescence dependence on lectin concentration at a fluorescence level of 1.5. The dependence of fluorescence intensity on the concentration of FITC-GSA was clearly observed at a fluorescence level of 6.5 for both bacterial cultures (Fig. 6). With FITC-WGA (Fig. 7), the greatest percentage of cells for both cultures had a 1.25 fluorescence level, with fewer cells at fluorescence

Fluorescence

FIG. 5. Fluorescence intensity of FITC-SBA-labeled cells of *A. brasilense*. NH₄⁺-assimilating (A) and N₂-fixing (B) cultures were examined with 10 (○), 30 (△), or 50 (□) μg of FITC-SBA.

Fluorescence

FIG. 6. Fluorescence intensity of FITC-GS I-labeled cells of *A. brasilense*. NH₄⁺-assimilating (A) and N₂-fixing (B) cultures were examined with 10 (○), 30 (△), or 50 (□) μg of FITC-GS I.

Fluorescence

FIG. 7. Fluorescence intensity of FITC-WGA-labeled cells of *A. brasilense*. NH₄⁺-assimilating (A) and N₂-fixing (B) cultures were examined with 10 (○), 30 (△), or 50 (□) μg of FITC-WGA.
levels of 3.5 and 6.5. At a fluorescence level of 6.5 with N₂-fixing cells, the percentage of cells with fluorescence increased with an increase in lectin concentration. With respect to fluorescence intensity, the binding of both FITC-WGA and FITC-SBA to cells yielded the highest percentages of cells at a fluorescence level of 1.25 or 3.5, while the binding of FITC-GS I and FITC-Con A to cells yielded the greatest numbers of cells at a fluorescence level of 6.5 or 3.5. Controls for these reactions were cells not exposed to FITC-lectins. No autofluorescence was observed with NH₄⁺-grown cells, while only 1% of the N₂-grown cells had a fluorescence level greater than 0.75.

Another parameter used to evaluate lectin interactions with bacteria was relative cell volume. The use of a 75-μm (pore size) orifice removed large bacterial clumps; however, small aggregates could enter the cytometer and be detected. The relative size of individuals detected by flow cytometry increased with exposure to lectins (Table 3). With N₂- and NH₄⁺-grown cells, a significantly larger volume was observed with all four FITC-lectins. Although the percentage of cells with volume larger than 1 did not differ greatly from that of the control, cells exposed to lectins yielded more individuals with larger volumes than did the controls. We attribute the increase in volume to cell agglutination caused by lectin-cell interaction.

The specificity of Con A and SBA for sugars on the cell surface of A. brasilense was demonstrated by the inhibition of lectin binding by appropriate sugars. The number of cells binding FITC-Con A significantly decreased when mannose was present, and the presence of galactose reduced the number of cells binding FITC-SBA (Table 4). Lectin binding by cells from both nitrogen cultures was similarly inhibited in the presence of hapten sugars.

**TABLE 4. Effect of sugars on FITC-lectins bound to cells of A. brasilense**

<table>
<thead>
<tr>
<th>Lectin added (30 μg)</th>
<th>Sugar added (0.1 μM)</th>
<th>% of cells from indicated culture binding lectina</th>
<th>NH₄⁺</th>
<th>N₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC-Con A</td>
<td>None</td>
<td>66</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-Mannose</td>
<td>69</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-Glucose</td>
<td>63</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>FITC-SBA</td>
<td>None</td>
<td>88</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-Galactose</td>
<td>63</td>
<td>33</td>
<td></td>
</tr>
</tbody>
</table>

* The number of cells counted by flow cytometry for each test was 10,000.

**Agglutination survey.** Lectin-dependent cell agglutination was observed with lectins with binding affinity for mannose, glucose, galactose, N-acetylgalactosamines, and N-acetylgalactosaminites (Table 5). No agglutination was observed with lectins which recognize fucose (LTA and U. europaeus agglutinin) or sialic acid (LFA). Except for the results of agglutination with S. tuberosum agglutinin, no apparent difference in lectin-cell interaction was seen with cells grown on either of the two sources of nitrogen.

Exopolysaccharide production by A. brasilense was influenced by the nitrogen source in the growth medium (Fig. 3). With no exopolysaccharide produced with N₂ but high levels produced with NH₄⁺. At this time, we do not know whether

**TABLE 5. Cellular agglutination of A. brasilense by lectins**

<table>
<thead>
<tr>
<th>Sugar specificity</th>
<th>Lectinb</th>
<th>Agglutination of cells from specified nitrogen sourceb</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-Acetyl-β-D-glucosamine and N-acetyl-α-D-glucosamine</td>
<td>GS-II</td>
<td>+</td>
</tr>
<tr>
<td>α-D-Galactose &gt; N-acetyl-α-D-galactosamine</td>
<td>WGA, GS-I</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-α-D-galactosamine &gt; N-acetyl-β-D-galactosamine</td>
<td>SBA</td>
<td>+</td>
</tr>
<tr>
<td>α-D-Mannose &gt; α-D-glucose</td>
<td>Con A</td>
<td>+</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>LFA</td>
<td>0</td>
</tr>
<tr>
<td>α-L-Fucose</td>
<td>UEA-I</td>
<td>0</td>
</tr>
<tr>
<td>α-D-Mannose &gt; α-D-glucose and influenced by fucose in oligosaccharide chain</td>
<td>LTA, LCH</td>
<td>+</td>
</tr>
<tr>
<td>β-D-Galactosyl(1 → 3)-N-acetyl-β-D-galactose</td>
<td>TKA</td>
<td>+</td>
</tr>
<tr>
<td>N-Acetyl-α-D-galactosamine</td>
<td>PNA</td>
<td>+</td>
</tr>
<tr>
<td>β-D-Galactosyl(1 → 4)-N-acetylgalactosaminosyl(1 → 2)-α-d-mannose</td>
<td>DBA</td>
<td>+</td>
</tr>
<tr>
<td>PHA-L</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

a No cells were found at the specified cell volumes of 2 and 4.

b Abbreviations: STA, S. tuberosum agglutinin; UEA-I U. europaeus agglutinin; L. culinaris agglutinin; TKA, T. kinlowii agglutinin; PNA, A. hypogaena agglutinin; DBA, D. biflorus agglutinin; PHA-L, P. vulgaris agglutinin.

+ Agglutination; 0, no agglutination.
exopolysaccharide binds the FITC-lectins or whether the capsule and exopolysaccharide are chemically identical. On the basis of the rate of cell growth, the production of exopolysaccharide, and the consistently high lectin-binding values, 24-h cultures seem most appropriate for further studies of cell surface evaluation.

DISCUSSION

The interaction of lectins with cells of A. brasilense was evaluated by fluorescence-activated flow cytometry and agglutination studies. The capsule of A. brasilense selectively bound lectins which had specificity for N-acetylgalactosamine, N-acetylgalactosamine, galactose, glucose, and mannos. The absence of significant binding by LFA and LTA suggests that fucose is not in the capsule. The binding of LFA to the capsule of A. brasilense is not clearly understood, because sialic acid is not commonly present in bacterial capsules. The binding by LFA to bacterial cells may reflect the presence of 2-keto-3-deoxyoctonate in the bacterial lipopolysaccharide. It has been reported that 2-keto-3-deoxyoctonate is recognized by LFA (8), and 2-keto-3-deoxyoctonate is characteristically present in the lipopolysaccharide layer of bacterial outer membranes. There was good agreement between the results of flow cytometry and agglutination studies of lectin-cell recognition. The only consistent response was the absence of agglutination between S. tuberosum agglutinin and bacteria from NH₄⁺ culture.

Considerable sensitivity was observed with flow cytometry. The fluorescence intensity of bacteria labeled with FITC conjugated to WGA, SBA, GS I, and Con A varied with the concentration of the FITC-lectin used (Fig. 2 to 5). The fluorescence level which gave the highest percentage of cells binding lectin varied with the specific lectin employed but was similar with respect to the use of NH₄⁺ or N₂ as nitrogen source. Volume analysis by flow cytometry of FITC-lectin interactions with bacteria revealed a marked increase in the percentage of aggregation with GS I and Con A of cells from NH₄⁺ culture. All FITC-lectins contributed to an increase in relative volume, although the percentage of cells with a volume larger than 1 did not increase with cells from N₂ culture.

Differences in the binding of FITC-lectins were readily apparent by fluorescence-activated flow cytometry. Although WGA and GS II have the same specificity for sugars, the percentage of cells binding FITC-GS II was markedly greater than that binding FITC-WGA. Also, there was a decline in FITC-lectin binding among 45-h cells from NH₄⁺ culture, compared with 24-h cells. With cells from N₂ culture, the percentage of cells binding lectins from a 45-h culture, compared with those from a 24-h culture, decreased significantly only with FITC-WGA. This difference between NH₄⁺- and N₂-grown cells at 45 h may be attributed to the copious production of exopolysaccharide by NH₄⁺-grown cells (Fig. 2). It is not known whether the capsule and exopolysaccharide of NH₄⁺-grown cultures have the same chemical content. Perhaps the cell washing procedure removed exopolysaccharide adhering to the capsule and accounted for the lower percentage of cells binding lectin from NH₄⁺-grown cultures. β-Hydroxybutyrate is produced by A. brasilense growing on N₂ (26), and our strain produced β-hydroxybutyrate; however, β-hydroxybutyrate in older cultures would not appear to contribute to a decline in the binding of FITC-lectins to cells, since none of the lectins used recognized β-hydroxybutyrate.

A change in cell surface antigens on cells of A. brasilense (originally called Spirillum lipoforum) associated with physiological activity was reported by Dazzo and Milam (6). They found that agglutination of cells by antisera specific for A. brasilense occurred at a higher dilution with 7-day cells than with 3-day cells. The reduction in the number of cells binding lectins at 45 h (Table 1) is consistent with the reported immune response (6), and future studies are planned to address the effect of culture age on the surface structures of A. brasilense.

Another parameter of FITC-lectin binding to bacteria which was examined was inhibition by lectin-binding sugars. Although complete inhibition of the binding of Con A and SBA to bacteria was not achieved with mannose and galactose, the partial reduction of lectin-cell interaction was consistent with the partial reduction of cell binding reported with other lectin systems (14). From these lectin studies, the capsule of A. brasilense seems to contain galactose; however, chemical analysis is needed for verification. Some strains of A. brasilense produce a poly-galacturonidase transglucosidase (34), and it would be important to learn whether the capsule could serve as a substrate for this enzyme or influence enzyme production. Although the binding of A. brasilense cells to plant roots is reported to be diminished in environments containing NH₄⁺, the capsule contents, as determined by lectin binding, of bacteria cultured in N₂ and NH₄⁺ appear to be similar. However, there is a difference in the extracellular polysaccharide synthesis of these two cultures, and it is important to direct attention to the characterization of extracellular polysaccharide synthesis in A. brasilense.

ACKNOWLEDGMENTS

We greatly appreciate the expert and patient technical assistance of Betty Albright in the use of the FACS analyzer.

This research was supported in part by a grant-in-aid for research by Sigma Xi and a GRAC-SRAC grant from the University of New Mexico.

LITERATURE CITED