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Transfer from *Rhizobium japonicum* to *Azotobacter vinelandii* of Genes Required for Nodulation

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A mutant strain of *Azotobacter vinelandii* that is unable to fix N_2 (Nif^-) was transformed to Nif^+ with DNA from *Rhizobium japonicum*. Of 50 Nif^+ transformants tested, 3 contained the O antigen-related polysaccharide that is present on the cell surface of a nodulating *R. japonicum* strain, but is absent from a non-nodulating mutant strain.

Recently, much attention has focused on the role that plant lectins play in the establishment of the N_2-fixing symbiosis between legumes and bacteria belonging to the genus *Rhizobium* (1-4, 10). Lectins from legumes interact with the O antigen-containing lipopolysaccharides of their rhizobial symbionts (10). The O-antigen fractions of wild-type and non-nodulating mutant strains of *Rhizobium japonicum* have been shown to differ in antigenic and chemical properties (6). Thus the O-antigen component of the lipopolysaccharides appears to play an important role in forming a successful N_2-fixing symbiotic association between *R. japonicum* and soybean plants.

Genes which seem to be involved in the initial stages of infection of white clover by *R. trifolii* can be transferred, by transformation (1), to mutant strains of *Azotobacter vinelandii* unable to fix N_2 (Nif^-). These genes control the synthesis of an *R. trifolii* surface receptor which binds to a clover lectin (4). Therefore, we decided to determine whether genes required by *R. japonicum* for nodulation could be transferred to *A. vinelandii*. The intergeneric transformation crosses were conducted as previously described (7), except that 2% sucrose replaced glucose in the transformation medium, and the donor strain was lysed in 0.1% sodium dodecyl sulfate. Wild-type *R. japonicum* 61A76 (obtained from J. Burton, Nitragin Co., Milwaukee, Wis.) was the donor, and *A. vinelandii* Nif^- mutant strain UW10 (8) was the recipient. The transformation frequency was 9.7×10^{-7} Nif^+ transformants per cell plated. The reversion frequency of strain UW10 was less than 3.3×10^{-8} per cell plated. The recipient and transformant cells appeared identical to each other and distinctly different from the donor cells in both size and morphology when examined by phase-contrast microscopy. These hybrid transformants also produced a green diffusible pigment characteristic of the recipient strain. Fifty of these Nif^+ transformant strains were tested for agglutination by rabbit antiserum prepared against whole cells of wild-type *R. japonicum* as previously described (6). Four of these transformant strains (Rj10-15, Rj10-19, Rj10-20, Rj10-49) gave a positive reaction and were studied further. Three different azotophages specific for wild-type *A. vinelandii* formed plaques on these four transformant strains, while three phages specific for wild-type *R. japonicum* did not form plaques on these transformants. The hybrid transformants grew aerobically in N-free medium (9) at 30°C with a generation time of 7.2 h. They also reduced acetylene to ethylene. Since the transformant strains retained many of the characteristic features of the recipient strains, it is apparent that they were not contaminants.

A non-nodulating mutant strain of *R. japonicum*, strain SM2, lacks a surface component that is present in the nodulating parent strain (6). The alteration in the cell surface of strain SM2 appears to reside in the O-antigen portion of the LPS. We wanted to determine whether the hybrid transformant strains contained the O-antigen determinant that is present in wild-type *R. japonicum* but absent from mutant strain SM2. To prepare an antiserum that would specifically detect the antigen unique to the nodulating strain, antiserum prepared against wild-type *R. japonicum* O antigen was adsorbed with whole cells of *A. vinelandii* and then with either strain SM2 whole cells or SM2 O antigen (6). This adsorbed antiserum agglutinated three of the four hybrid strains as well as wild-type *R. japonicum* (Table 1). Neither wild-type *A. vinelandii*,

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TABLE 1. *Agglutination of hybrid Nif⁺ transformants with anti-O-antigen antiserum*[a]

Strain	Description	Agglutination reaction with antisera adsorbed with[b]: UW10	UW10 and SM2	UW10 and SM2 O antigen	Wild-type *R. japonicum* O antigen
A. vinelandii					
UW10	Nif⁻ (8)	−	−	−	−
UW10r	Nif⁺ revertant of strain UW10	−	−	−	ND
RtAv10-54	Nif⁺ transformant from *R. trifolii* × *A. vinelandii* cross (1)	−	−	−	ND
Rj10-15	Nif⁺ transformant from *R. japonicum* × *A. vinelandii* cross	+	±	−	−
Rj10-19	Same as Rj10-15	+	+	±	ND
Rj10-20	Same as Rj10-15	+	+	+	−
Rj10-49	Same as Rj10-15	+	+	+	−
R. japonicum					
Wild type		++	+	+	−
SM2	Non-nodulating mutant strain (5)	++	−	−	−

[a] Preparation and adsorption of antisera were conducted as previously described (6).

[b] Agglutination reactions were conducted as described (6) except that the reactions were scored after 8 h of incubation. ++, Very strong agglutination; +, strong agglutination; ±, weak agglutination; −, no agglutination; ND, not determined. All agglutination evaluations were compared with cell autoagglutination controls lacking antiserum.

strain UW10, strain UW10r (a spontaneous Nif⁺ revertant of strain UW10), wild-type *R. trifolii*, strain SM2, or strain RtAv10-54 (a hybrid transformant containing the capsular polysaccharide from *R. trifolii*) was agglutinated with the antiserum containing antibodies against the O antigen of *R. japonicum*. Serum with O-antigen antibodies was adsorbed with O antigen from the wild-type *R. japonicum*, and the resulting adsorbed serum no longer agglutinated wild-type *R. japonicum* or hybrid strains Rj10-20 or Rj10-49. Binding of SM2 O-antigen-adsorbed antiserum to cells of the hybrid strain Rj10-49 was confirmed by indirect immunofluorescence (4) (Fig. 1). Results with strain UW10r were negative (no fluorescence). It seems, therefore, that the hybrid strains Rj10-19, Rj10-20, and Rj10-49 contain antigenic determinants (presumably as part of the O antigen) on their cell surface which are present on the cell surface of wild-type *R. japonicum* but not on the surface of strain SM2, the non-nodulating mutant strain. Hybrid strain Rj10-15 does not appear to carry the antigenic determinant unique to wild-type *R. japonicum*. In addition to strain SM2, all of the serological experiments in Table 1 were performed with another non-nodulating *R. japonicum* mutant strain, SM1 (5), yielding the same results observed with strain SM2. The wild-type *R. japonicum* and strains SM1 and SM2 had identical colony morphologies on a variety of media.

The binding of *R. japonicum* to soybean roots may be a prerequisite for infection and subsequent nodulation. Transfer of *Rhizobium nif* genes (W. J. Page, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, K82, p. 199), as well as genes specifically required for the infection process by *Rhizobium*, to *A. vinelandii* should be useful for studying these genes in a system that does not involve the plant host.

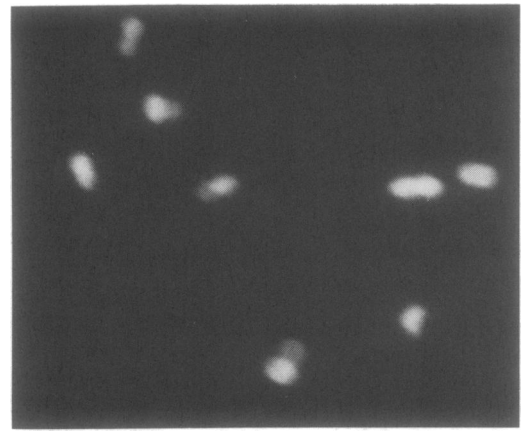

FIG. 1. *Immunofluorescence of the hybrid strain Rj10-49 after treatment with rabbit anti-O-antigen antiserum and fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulins. The antiserum was previously adsorbed with strain SM2 O antigen as described (6). Fluorescence microscopy was performed as previously described (4). Controls with strain UW10r gave no fluorescence under these conditions.*

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