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ADSORPTION OF RHIZOBIA TO CEREAL ROOTS

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Summary

The attachment of Rhizobium japonicum 61A89 to the roots of wheat and rice seedlings is an equilibrium process that follows a Langmuir adsorption isotherm. This model predicts a maximum of 8 x 10^9 viable bound bacteria per g of root. Different strains of bound rhizobia have both characteristic appearances and surface densities on the root surface. The bound rhizobia did not fix nitrogen.

Symbiotic nitrogen fixation in the major agronomic crops is limited primarily to the legume family and does not occur in cereals such as wheat or corn. One approach to the induction of nitrogen fixation in cereals is to attach large numbers of nitrogen-fixing bacteria to cereal root surfaces. The surface area of plant roots is extensive and bacteria are able to bind to roots both specifically[1] and non-specifically.[2] Large numbers of nitrogen-fixing bacteria adhering to the root may provide much of the host plant's requirements for ammonia.

We have begun to quantitatively examine the binding of nitrogen-fixing bacteria to the roots of wheat and rice. Several species of rhizobia bind to these roots in extremely large numbers. The number of rhizobia adhering to the root under hydroponic conditions is a function of the concentration of rhizobia in the medium surrounding the root. The binding is analyzed in terms of a dynamic equilibrium model, that is, a Langmuir adsorption isotherm.[3]

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Materials and Methods

Wheat, Triticum aestivum L. var. Chris, and rice, Oryza sativa CSM-3, seeds were surface sterilized with ethanol and 5% sodium hypochlorite and germinated under aseptic conditions at room temperature. The seedlings were grown in hydroponic culture at 22°C in a medium containing 25% modified Hoagland's nutrient solution and 10% yeast extract-mannitol solution[4] without $CaCO_3$. The rhizobia, Rhizobium japonicum 61A89, Rhizobium phaseoli 127K17, and Canavalia ensiformis spp. 22A3, were maintained on yeast extract-mannitol agar slants and grown at 30°C in shake flasks containing yeast extract-mannitol solution. Typically, 1 to 2 week old seedlings were inoculated with aliquots of rhizobia suspension and incubated at 22°C. Bacterial binding was detected both by scanning electron microscopy and by a viable cell count of a crushed root suspension.

Results and Discussion

Viable cell count experiments indicate that the roots of a 2 week old wheat seedling can bind up to 10^9 rhizobia. The maximum numbers of viable rhizobia which we have observed bound per g of wheat root are: 5.9×10^9 for 61A89; 1.3×10^9 for 127K17; and 0.6×10^9 for 22A3. The density of viable cells in the immediate vicinity of the root is much greater than that in the surrounding medium for strains 61A89 and 127K17. At low concentrations, the number of attached cells per g of root is a factor of 7 greater than the number of unattached cells per ml of solution.

The number of rhizobia bound per g of root is directly proportional to the concentration of unattached rhizobia at low concentrations. At higher concentrations, the binding becomes non-linear. The adhesion of viable rhizobia to the roots can be analyzed in terms of an equilibrium model which assumes that the bacteria adsorb to a fixed number of equivalent, independent sites on the root surface and that the bound and free bacteria are in dynamic equilibrium. A Langmuir adsorption isotherm of the form

$$C_b = nKC_s/(1 + KC_s) \qquad (1)$$

describes the binding. C_b is the concentration of rhizobia adsorbed to the root in numbers of viable cells per g of root; C_s is the concentration of rhizobia in suspension with the root in numbers of cells per ml of solution; n is the maximum concentration of adsorbed rhizobia; and K is the association constant for the equilibrium binding. The adsorption of R. japonicum 61A89 to both wheat and rice roots is illustrated in Figure 1. Values of n and K, for 61A89, determined graphically from the intercepts of a plot of C_b^{-1} vs. C_s^{-1}, are $8 \pm 3 \times 10^9$ g^{-1} and $1 \pm 0.3 \times 10^{-9}$ ml, respectively. We have observed a slow, time-dependent dissociation of the rhizobia from wheat roots. This dissociation would be expected in an equilibrium model.

Scanning electron microscopy shows that each of these rhizobia strains has a characteristic appearance when adsorbed to the wheat root surface and that they cover the surface with little overlap. In Figure 2A, 61A89 is uniformly distributed over the root surface and is adsorbed predominantly, but not exclusively, with its long axis parallel to the surface. Transmission electron microscopy indicates predominantly polar binding for 61A89. Possibly, the absence of end-on binding as seen with scanning electron microscopy is a fixation artifact. In Figure 2B, 127K17 binds in a polar or end-on manner and is attached to the root surface by fibers or filaments.

The surface density of the adsorbed rhizobia, as determined by scanning electron microscopy, correlates very well with concentration of bound rhizobia in the viable cell count experiments. Wheat roots were prepared for scanning electron microscopy at intervals of 1 to 6 days after the seedlings were inoculated with rhizobia, and the density of the bound rhizobia on the root surface was determined by counting the number of bacteria on random areas of the root having a total area of approximately $6000\mu^2$. The surface density of each rhizobia strain increased

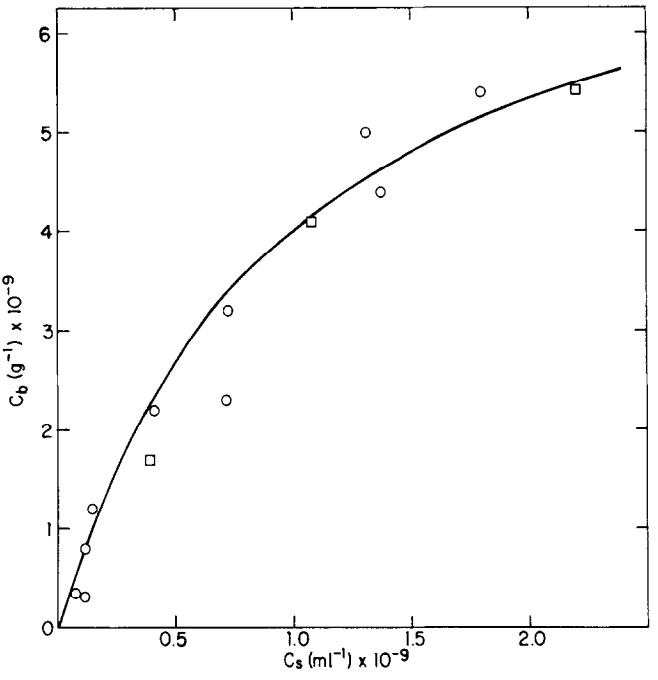

Figure 1. The concentration of bound R. japonicum 61A89, C_b (g^{-1}) is plotted as a function of the concentration of free rhizobia, C_s (ml^{-1}), for wheat (○) and rice (□) roots in hydroponic culture at 22°C. In 3 separate experiments, the seedlings were inoculated with 2 ml of a 61A89 suspension in yeast extract-mannitol solution having a cell density ranging from 0.3 to 1.1 x 10^9 ml^{-1}, and incubated for a period of 2 to 7 days. The roots were excised, washed in phosphate buffered saline (PBS) to remove unattached bacteria, and crushed in a tissue grinder containing 1 ml of PBS. Both the crushed root suspension and the hydroponic growth solution containing free rhizobia were diluted in PBS and plated on yeast extract-mannitol agar. Viable colonies were counted after 1 week at 30°C. The data points are derived from 2 experiments with wheat, performed at separate times and on different batches of seedlings, and 1 experiment with rice. The bound and free concentrations for 61A89/wheat are the average of values for two roots and solutions counted simultaneously. The curve is calculated from the adsorption isotherm for n=8 x 10^9 g^{-1} and K=1 x 10^{-9} ml.

with the time of incubation as did the concentration of adsorbed cells in separate viable cell count experiments. After a 6-day incubation, the surface density of 61A89, 3.5 x 10^7 cm^{-2}, was significantly greater than that of 127K17, 4.8 x 10^6 cm^{-2}, and

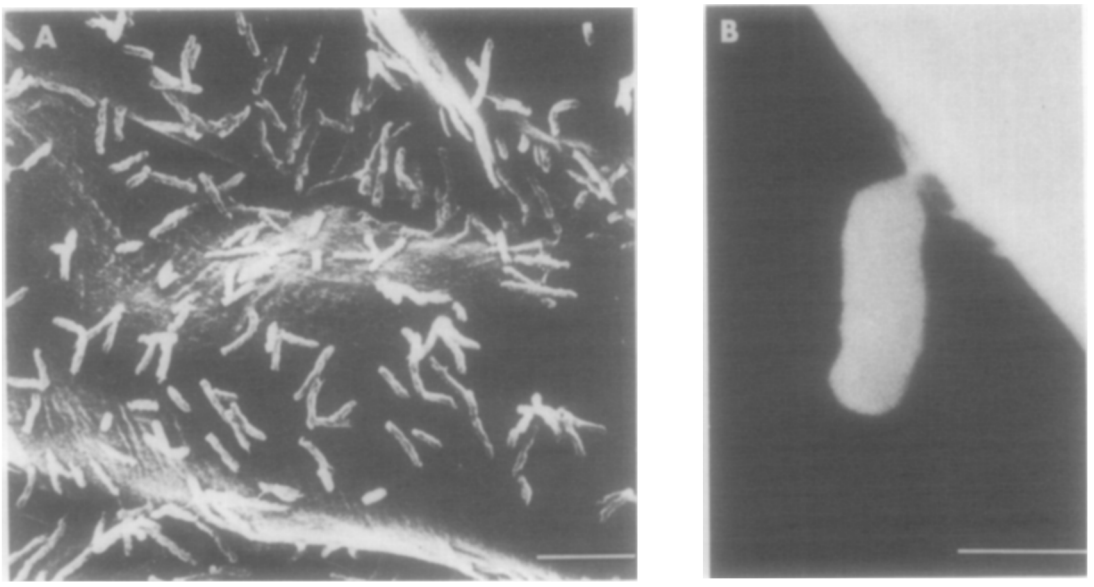

Figure 2. Scanning electron micrographs illustrate rhizobia adhering to wheat roots. A) R. japonicum 61A89 covers the root surface uniformly. B) R. phaseoli 127K17 is attached end-on to a root hair by fibers or filaments. The bars indicate 3.0 and 0.5μ for A and B, respectively. The samples were fixed in buffered 5% glutaraldehyde overnight, rinsed in Millonig's phosphate buffer, pH 7.0, and dehydrated through an ascending series of ethanol solutions into dry acetone. The roots were then critical point dried by substitution of the acetone with liquid CO_2. The dried samples were rotary shadowed with gold/palladium and examined on a ETEC Autoscan.

22A3, 3.2×10^6 cm^{-2}. By comparison, viable cell counts after 5 days gave bound concentrations of 2.2×10^9 g^{-1} for 61A89, 5.9×10^8 g^{-1} for 127K17, and 3.1×10^8 g^{-1} for 22A3. We can estimate that the maximum surface density of the rhizobia on the roots would be on the order of 10^8 cm^{-2}. After 6 days, 61A89 covers approximately one third of the root surface and occupies approximately the same fraction of available binding sites.

The surfaces of both wheat and rice roots appear quite similar to the adsorbing rhizobia since the adsorption isotherm for 61A89 binding to each is identical. The rhizobia bind to

other surfaces, but not to the degree that they do to the root surfaces. For example, the surface density of 61A89 on cotton fibers after a 6-day incubation is one to two orders of magnitude less than on wheat roots. Possibly, both the bacteria and the roots produce the components required for extensive adsorption.

With the aerobic growth conditions used, no significant nitrogen fixation activity, as measured by acetylene reduction,[5] was observed for the adsorbed bacteria. The washed, excised roots of wheat seedlings, growing with 61A89 for 6 days under aerobic conditions, were placed in 1 ml of CS7 medium[6] and incubated for 2 hr at 30°C under 10% C_2H_2/air, 10% C_2H_2/5% O_2/85% Ar, and 10% C_2H_2/1% O_2/89% Ar. An upper bound to the acetylene reduction rate for the adsorbed 61A89 is 20 pmol/hr/root or an estimated 4 pmol/hr/10^8 cells.

The time and temperature dependence of rhizobia attachment and detachment as well as the binding of other nitrogen-fixing bacteria is currently being examined.

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