The Use of Isolated Root Cap Cells to Teach Cellular Aspects of Host-Parasite Recognition

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Meristematic cells in the root tip daily produce hundreds of peripheral root cap cells that envelop each root and constitute a major component of the surrounding rhizosphere (2,14). The freshly detached cells from many species, including legumes and cereals, are more than 90% viable (6,8,10). The cells can survive for long periods in culture, and they can be induced to divide and grow into callus (1,4,8). More important, the sloughed cells can survive for some time in the soil (17) and are capable of responding to fungal infection by producing papillae (15).

Suspensions of isolated root cap cells provide a useful tool for demonstrating cellular interactions between plants and pathogens (3,5–11). Peripheral root cap cells become detached from the root so easily that they are lost during most preparative laboratory techniques; the cells slough from the root within seconds of exposure to water. This gentle isolation procedure leaves cell walls intact, so that an osmoticum is not needed to maintain cell viability. Furthermore, harvesting root cap cells does not damage the root, which simply generates a new supply. It is therefore possible to compare cellular responses with whole plant reactions: The cells from a single plant can be used to observe microbial behavior, and the same plant can be inoculated to evaluate susceptibility.

Root cap cells have been used in simple procedures to provide reproducible, quantitative data in assays of plant sensitivity to host-specific fungal toxins, bacterial binding, and fungal and bacterial chemotaxis (3,5–12). We recently found that zoospores of an isolate of *Pythium dissotocum* are specifically and exclusively attracted to the root cap region of cotton roots (3,7). Furthermore, the zoospores encyst, penetrate, and kill isolated root cap cells within minutes. The ability of such soilborne pathogens to recognize and colonize sloughed root cap cells suggests that these living cells may play an unrecognized role in rhizosphere interactions. In laboratory classes, I have found that root cap cells provide an effective way to illustrate cellular responses to microorganisms and to correlate such effects with whole-plant pathogenesis.

Binding of *Agrobacterium tumefaciens* to host cells is believed to be a prelude to T-DNA transfer and tumor induction (13). Binding of the bacteria to root cap cells is strongly correlated with susceptibility to the crown gall bacterium (9). Thus, mutants or strains of *A. tumefaciens* that do not bind to cells of a particular plant do induce tumor formation on the plant. Conversely, plants whose cells do not bind a given Ti-plasmid containing *Agrobacterium* strain do not form tumors in response to inoculation. For example, biotype 1 strain B6 binds to pea root cap cells and causes tumors on pea but does not bind or cause tumors on oats. Biotype 3 strain Ag63 binds to and infects grape tissue (16) but does not bind or cause tumors on pea or oats. The following exercise describes the use of isolated cells to demonstrate the correlation between binding and susceptibility.

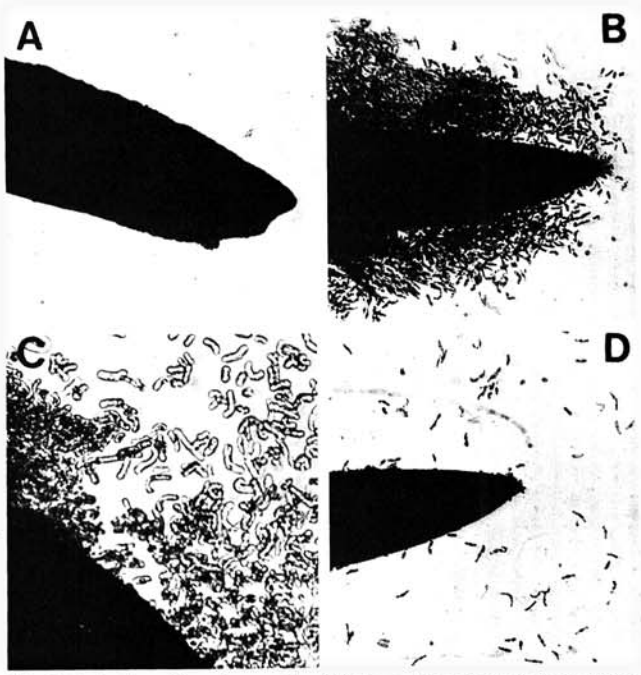

Fig. 1. Isolation of root cap cells. **(A)** Cone-shaped root of a seedling (cotton) germinated for 2 days on water agar overlaid with filter paper. **(B)** The same root tip 60 sec after adding a 100-µl droplet of distilled water. **(C)** At higher magnification. **(D)** After agitating the water droplet. (Reproduced with permission from *American Journal of Botany* 73:1466-1473)

Isolation of root cap cells

Surface-sterilize seeds of oats and pea (Little Marvel, Progress No. 9, Target, and Thomas Laxton are examples of highly susceptible pea varieties that are commercially available) by immersion for 5 min in 95% ethanol and 5 min in 0.12% sodium hypochlorite (50% commercial bleach); rinse four or five times with sterile water. Germinate seedlings on 0.7% water agar overlaid with sterile filter paper. IMPORTANT: Roots must not be allowed to penetrate the agar or to be immersed in free water, or the cells will be lost.

Root cap cells can be isolated when radicles are 5–15 mm long, after 2 or 3 days at 25–27 C. For each binding assay, collect cells from five to eight pea radicles and eight to 10 oat radicles. The isolation process can be monitored with a dissecting microscope (Fig. 1). Immerse the root tips (cells from different plants can be pooled for the binding assay) into a 300-µl droplet of sterile distilled water for 30–60 sec, then agitate the water by taking it up with a pipette and releasing it several times. Yields will vary with species; legumes and cereals yield approximately 1,000–3,000 cells per root (8). Most cells will be separate; small clumps that occur with some genotypes can be eliminated by filtering through a 30-µm mesh screen (optional). SAVE THE SEEDLINGS.

Testing cell viability

Viability of the cells can be assessed by any of several methods. In many but not all cases, dead cells can be readily distinguished from living cells by an obvious loss of cell structure and collapse of the cytoplasm. Staining with fluorescein diacetate (12) is the most rapid and objective test,

but staining with neutral red and/or observation of cytoplasmic streaming are satisfactory if a fluorescent microscope is not available. Store fluorescein diacetate (0.5% w/v) in acetone at 0 C. Place a 1- to 5-μl droplet onto a microscope slide and allow the acetone to evaporate before adding 20–25 μl of plant cell suspension; do not stir. Cells with intact membranes will take up the stain and begin to show bright yellow-green fluorescence within 5 min. Neutral red can be stored as an aqueous solution and added directly to plant cell suspensions at a final concentration of 0.1%. The stain accumulates by binding to phenolic components within vacuoles of living cells, which show a light pink to red color within 10–15 min. Dead cells do not accumulate the stain, but some binding of the stain to cell walls can give the appearance of cells that are outlined in red. Cytoplasmic streaming can be seen more readily if stimulated by agitating cells in a vortex mixer for several seconds before observation. Viability can be confirmed by testing the ability of cells to undergo plasmolysis in the presence of an osmoticum, such as 0.6 M sucrose. If care has been taken to protect roots from drying, 90–95% of the cell populations from most seed lots should be viable.

Binding assay

Divide each plant cell suspension into three 100-μl samples in wells of a microtiter plate. *A. tumefaciens* strains B6 and Ag63 (available from ATCC) are cultured overnight on Luria broth or other rich medium (liquid or solid media can be used); bacteria should be diluted directly into water without washing, and cell concentrations estimated turbidimetrically. Although the exact concentration is not critical, a range between 10^7 and 10^8 cfu/ml (absorbance of approximately 0.047–0.47 at a wavelength of 625 mμ) of water is easiest to evaluate; the same concentration should be used in all treatments. Add 100 μl of each bacterial suspension to a root cap cell sample and stir; add 100 μl of water to the third sample. Incubate the mixtures at room temperature for 60–90 min. Gently take up and release the suspension with a pipette several times to mix, then immediately place a 20-μl droplet onto a microscope slide and add a coverslip (root cap cells rapidly settle out of suspension).

Check the bacteria-free control treatment for contamination at 100–400× magnification (phase contrast or interference contrast optics are helpful but not necessary); root cap cell edges should be smooth and free from bacteria. In the test samples, differences in binding should be apparent even without washing away unbound bacteria. Bacterial stains are not required to visualize binding. In positive reactions, a higher concentration of bacteria will be present on the root cap cell surface or within the surrounding polysaccharide exudate (Fig. 2A). In negative or reduced binding reactions, the bacteria may be evenly distributed throughout the suspension of plant cells (Fig. 2B), or the bacterial density may be reduced near the cell surface (Fig. 2C). Cells can be washed to some extent by adding water and drawing the excess off with a tissue at the edge of the coverslip. However, suspended bacteria can get trapped against individual plant cells as the slide dries. Responses are clearer if the root cap cells are washed on a 10-μm mesh screen to eliminate unbound bacteria before examination (Fig. 2D). Most pea cells will bind dozens of cells of *A. tumefaciens* strain B6 (Fig. 2A and D) but few or no cells of strain Ag63 (Fig. 2B). Most oat cells will be bacteria-free in both treatments, with no more than two bound bacteria visible on any cell (Fig. 2C).

Disease response

Inoculate the same seedlings whose root cap cells were used in the binding assay. Puncture the root several times with a dissecting needle or scalpel and immerse the seedling in several milliliters of bacterial inoculum for 5 min. Inoculate five or six seedlings of each plant species with each of the *A. tumefaciens* strains and plant in growth pouches or in vermiculite or soil in individual pots. Keep the plants moist but not wet with nutrient solution or water. Evaluate the inoculation sites for the development of crown gall tumors after 10 days to 2 wk.

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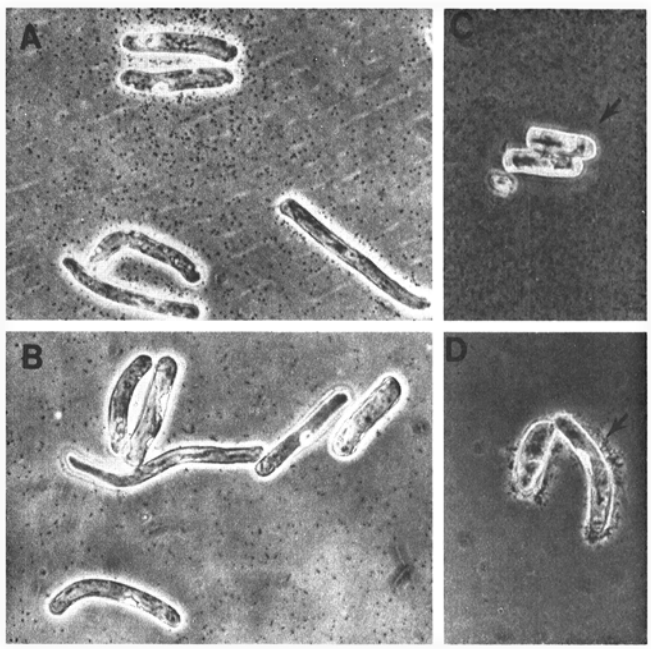

Fig. 2. Interactions of *Agrobacterium tumefaciens* with isolated root cap cells. **(A)** Increased density of bacteria around periphery of isolated pea root cap cells (unwashed) 2 hr after adding *A. tumefaciens* strain B6 (approximately 5×10^7/ml). **(B)** Uniform distribution of *A. tumefaciens* strain Ag63 (approximately 5×10^7/ml) throughout suspension of isolated pea root cap cells (unwashed), after a 2-hr incubation. **(C)** Decreased density (arrow) of bacteria around periphery of oat root cap cells (unwashed) 2 hr after adding *A. tumefaciens* strain B6 (approximately 10^8/ml). **(D)** Masses of bacteria (arrow) bound to pea root cap cells. Root cap cells were incubated for 2 hr with *A. tumefaciens* strain B6 (approximately 10^8/ml) and then washed on a 10-μm mesh screen to eliminate unbound bacteria from suspension. (Reproduced with permission from *Plant Cell Reports* 6:287-290)