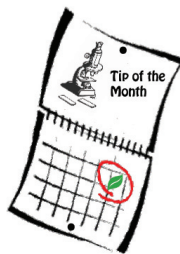


# Diagnostic Updates



## Peppers as an Alternative to Potato Slices for Bacterial Soft Rot Bioassay

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A very useful tip I picked up from bacteriologist and pepper pathologist Dr. David Ritchie was to use pepper fruit to screen for soft rot activity in bacteria. This test is easier to set up and to read than the traditional potato slice test. I can vouch for its effectiveness with *Pectobacterium (Erwinia) carotovora*. It should work for *Dickeya (Erwinia) chrysanthemi* as well, but I don't know how other less pectolytic bacteria such as *Pseudomonas viridiflava* would behave.

- 1) Obtain ripe pepper fruit. I use bell pepper but suspect that other types will work as well. Peppers can be store-bought; surface traces of fungicides should not interfere with the test.
- 2) Wash the fruit by hand in mild dish detergent.
- 3) If you are concerned about ambient bacterial contamination, spray the fruit to runoff with 70% ethanol and allow it to dry. After this point, handle the fruit only from the ends.
- 4) Set the fruit on its flattest side, so it is unlikely to roll. It will try to, anyway, so be careful.
- 5) Using a permanent marker, draw 1- to 2-cm circles on the upper side of the fruit to mark the inoculation points. Make duplicate inoculations for each bacterium you want to test plus one for the negative control and one for a positive control, if you have one. Label each circle to indicate what culture or control will be inoculated there.
- 6) Use a dissecting needle to pick a lock, or else mistreat it in some other way so that it acquires a small hook at the tip (Fig. 1). This instrument is better at breaking through the pepper skin than a toothpick, and it can pick up more bacteria than a straight needle.
- 7) Use the flamed and cooled needle to lift a visible amount of material from a log-phase bacterial colony, and puncture-inoculate the fruit at the center of the corresponding circle. Try not to break through into the locule of the fruit. If you did not introduce many bacteria in the process, you can pick up a few additional cells and place them in the hole.
- 8) Use sterile water to inoculate the negative control circle in the same way. You could also use a nonpectolytic bacterium like *Pseudomonas syringae*.
- 9) Place the fruit on the bottom (or overturned lid) of a disposable Petri dish, within a moist chamber. Close the chamber and incubate at room temperature for 18 to 24 hours.
- 10) A darkened, flattened area at the point of inoculation (Fig 2) indicates soft-rot activity. The darkened area will be extremely soft when probed. If nothing has occurred by 24 h, the result should probably be read as negative, though I've been known to give it a little longer.
- 11) Dispose of the pepper/bacteria cocktail appropriately. 🌿



Fig. 1. A "modified" dissecting needle useful for inoculating bacteria into pepper fruit. Small tick marks are millimeters.



Fig. 2. Positive (left) and negative (right) results of the bacterial soft rot test on pepper. Photo taken about 30 hours after inoculation.