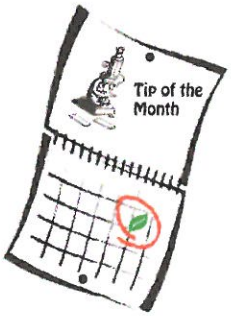

Tobacco Hypersensitivity; the First Test to Screen Bacteria for Pathogenicity

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Tobacco hypersensitivity is a fast and convenient way to screen bacterial cultures for pathogenicity. It works particularly well for *Pseudomonas* but can be variable for *Xanthomonas* and *Ralstonia*. Some Xanthomonads may require some tweaking of the environmental conditions the tobacco is grown in (Fahy and Persley, Lelliott and Stead), and the response may take up to four days (Klement and Goodman). *Erwinia amylovora* and some of the coryneform bacteria will also cause a hypersensitive response. *Ralstonia solanacearum* cause various results depending on the race. Race 1 results in chlorosis after two days, race 2 induces a typical hypersensitive response in one day and race 3 results in chlorosis after two to eight days (Lozano and Sequeira).

You will need to have tobacco plants and access to a greenhouse. We seed the tobacco in a small tray and transplant them into larger pots. Plants should be a healthy green color and preferably not flowering but flowering plants will usually work fine.

Prepare an aqueous suspension of the bacterium of about 108-1010 CFU/ml from a 24 to 48 hr culture. We don't actually determine the CFU's but the suspension should be turbid. Most references will suggest that you use a needle to inject the cell suspension but we find it easier to simply hold the barrel of the syringe against the bottom of the leaf as illustrated in figure 1.

Fig. 1. Injection of a bacterial suspension through the bottom of a tobacco leaf; note the water-soaked tissue.

It is a good idea to use a positive control when carrying out this test; water can be used as a negative control.

Turn the leaf over, bottom up, hold the needle or syringe

Fig. 2. Isolate "1" was tobacco hypersensitive negative and isolate "2" (three patches) was tobacco hypersensitive positive.