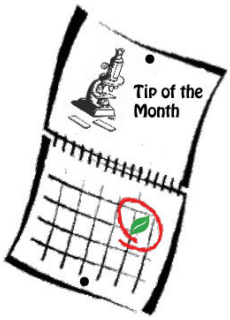

Tobacco Hypersensitivity; the First Test to Screen Bacteria for Pathogenicity

Dr. Robert Wick, University of Massachusetts, Department of Plant, Soil and Insect Sciences

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.

barrel against the leaf and lightly press with your index finger of your other hand on the leaf opposite the syringe. Gently force the suspension into the mesophyll. If the stomata are closed, it can be difficult to get good infusion. A water-soaked area will develop as the suspension infiltrates the mesophyll (see figure 1). Generally the entire water-soaked area will become necrotic within 12 to 24 hours. Non-pathogenic bacteria will not result in any symptoms; occasionally non-pathogens result in chlorosis.

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NPDN-USDA APHIS 2010 Last Bioinformatics Advanced Diagnostic Training for this Summer

Karen L. Snover-Clift, Cornell University and Laurene Levy, USDA-APHIS-PPQ-CHPST-NPGBL

The NPDN Diagnostics Program Area Committee and members of USDA-APHIS-PPQ-CHPST-National Plant Germplasm and Biotechnology Laboratory (NPGBL) are continuing to offer training sessions on Bioinformatics this summer. The last session with available spaces is offered on August 25 starting at 1pm, August 26 and 27. The 2 ½ -day session will cover analysis of obtained sequences from both plus and minus strands, editing sequences, blasting sequences, understanding blast results based on size and gene target, when to directly sequence PCR products or clones, which genes are used for sequence analysis for fungi, bacteria, and viruses, what sequence analysis programs are available commercially or as freeware, and hands-on use of sequence analysis programs using sequences from case studies for different pathogen types. Participants of this meeting are expected to cover their travel, lodging and meal expenses. There is no registration charge for the meeting or for meeting materials. These expenses are covered by our colleagues at USDA-APHIS-PPQ-CPHST-NGBTL. If you are interested in participating in any of these workshops please contact Karen Snover-Clift at kls13@cornell.edu.

APHIS Job Announcement

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