Plant Pathogenic Bacteria
A Basic Guide to Pathogenicity Testing

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Main types of pathogenicity tests

- Hypersensitive Response [HR] – a test for a necrosis induced response of tobacco leaves infiltrated with the test organism
- Host tests
  - Single host tests – to confirm pathogenicity against a specific host
  - Multiple host tests – to confirm pathogenicty against an array of host species or cultivars as support pathovar/biovar and race determinations, respectively

General principles to pathogenicity tests

- Be clear on the question you wish to address. Consider:
  - Confirmation on having isolated a bacterium that is pathogenic to plants – can be achieved by the HR test
  - Confirmation of pathogenicity [Koch's postulate] on a particular host – requires testing against only the host of isolation or an acceptable alternative
  - Confirmation of a pathovar / biovar status – will require an extended range of host species
  - Confirmation of race – will require an extended range of cultivars of a single host species

Hypersensitive response [HR]

- The HR tests provides a rapid test for the presence of most, but not all, plant pathogenic bacteria
- Simplistically, the HR response is triggered by the infiltration of the inter-vainel lamella of tobacco leaf with your test organism and the recognition by the host plant of the presence of pathogenicity genes, triggering a necrosis of plant cells

Hypersensitive response – worked example

- Inoculum should be approx. 10^3cfu ml^{-1} [just clouded]
- HR reaction will be evident within 24 – 48hrs as a necrotic zone
- The control [infiltration by sterile water] will remain normal
- Various methods have been described for the infiltration
- Simplest is to place a syringe [without needle] over a single pin-pricked leaf hole, with a finger covering the other side, and gently forcing the inoculum through

Koch's postulate

- Confirmation of a successful isolation is only proven when Koch's postulate is satisfied:
  - When the isolated organism is inoculated back into the same host species from which it was isolated [or an acceptable alternative if this is impracticable] and is seen to cause the same disease symptoms as initially described for that organism
  - When the inoculated organism is re-isolation [assuming it to be cultureable] and shown to have the same diagnostic properties as the original inoculated isolate
  - When control plants contained within the same experiment unit as the inoculated plants [glasshouse etc] are shown not to exhibit any of the disease symptoms as the inoculated plant and are otherwise free of any potential plant pathogenic organisms

The nature of pathogenicity and host tests

- Pathogenicity tests tend to have variable success, especially when using cultures derived from culture collections and of longterm preservation
- Success should be greater with freshly isolated bacterium as provided through a recent isolation
- There is no set protocol for a pathogenicity test; it’s on a case-by-case basis and influenced by the facilities you have available
- However, there are some guiding principles that will improve success

General principles to host tests [1]

- Value the information you have to hand
  - Symptoms – this directs where and how the inoculm may best be applied:
    - Leaf spots and stem cankers – a mist treatment to leaves and stems, with some physical wounding
    - Wilt – a root drench, with a root cut; or a stem injection
    - Gall – a stem injection
    - Soft rots of fruits and tubers – a drench treatment, with physical wounding
  - Undertake a literature search as to proven methods for pathogenicity testing as seem appropriate to the type of organism you have; noting symptoms and host

General principles to host tests [2]

- Think through the three stages for testing
  - Experimental design and implementation
    - Need for test plants
    - Need for control plants
    - Need for replication for both test and control plants
    - Need for randomisation of treatments
  - Post inoculation and cultivation of the plants
    - Light cycle, temperature and humidity need to be conducive to plant growth and disease symptom expression
    - Humidity may be increased for short period [12-24hrs] post inoculation if a mist treatment to promote infection
  - Re-isolation of the inoculated organism from disease symptoms observed
    - Applicable when aiming to confirm Koch's postulate