

## PLANT PATHOLOGY - Demonstrating Koch's Postulates

Robert Koch was a bacteriologist who lived from 1843 to 1910. In his work on diseases, he was trying to establish whether a microbe, obtained from a diseased patient, was in fact the cause of the disease. This led him to formulate 'Koch's Postulates' – a set of conditions which need to be fulfilled to establish which organism is causing a particular disease.

Many *plant* pathogens are safe to use in a school or college laboratory (but simple safety precautions must be observed – see safety notice below). If you attempt to isolate a pathogen from infected fruit, vegetable, or other plant tissue, you are likely to obtain a plate with several organisms growing on it. This is because, once a plant becomes weakened by disease, secondary saprophytic organisms often invade. So, when identifying which is the disease-causing organism, the first step is to demonstrate Koch's Postulates.

To demonstrate Koch's Postulates, you must do the following.

- (i) Describe and record the symptoms shown.
- (ii) Isolate the suspected pathogen from the infected plant material and establish a pure culture.
- (iii) Use the pure culture to infect new plant material.
- (iv) Describe and record the symptoms shown by the new plant. Check that these are the same as your original observations.
- (v) Re-isolate the organism. Check that this is the same as that isolated previously.

This protocol allows students to demonstrate and test Koch's Postulates, using apples infected with the fungus *Penicillium expansum*. The required experimental work extends over 3 to 4 weeks, but the essential steps can be demonstrated in a single practical session if students are provided with cultures and infected fruit which represent different stages in the sequence of steps.

### Preparation for the practical work

Agar plates and apples need to be inoculated about one week before the first practical session and incubated at 20 to 25 °C. When inoculating agar plates from infected apples, it is best to push the piece of infected tissue right into the agar jelly in the centre of the plate. Plates should be inoculated the right way up so need to be poured in a way that gives very little condensation.

Each group is provided with 4 apples

- ▶ apple 1 – wounded and inoculated 7 days previously with *Penicillium expansum*
- ▶ apple 2 – 'control' apple – on one side this is wounded and inoculated with sterile distilled water, on the other side a fungal culture is applied to the intact skin (without wounding)
- ▶ apples 3 and 4 – fresh apples, to inoculate

Each group also has

- ▶ one Petri dish (plate) with a 7-day old culture of *Penicillium* on malt agar
- ▶ two clean malt agar plates, micropore tape, scalpel, Bunsen burner, methylated spirits, ruler

In carrying out the steps in the protocol, always work with the 'control' apple *first*, to avoid cross contamination.



#### SAFETY NOTICE

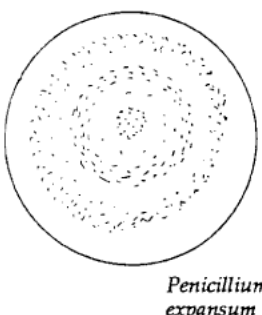
- Ensure that bench surfaces are non absorbent.
  - Swab bench with disinfectant before and after practical work.
  - No eating, drinking or chewing in the laboratory.
  - Wear a laboratory coat or apron.
  - Keep lids on cultures when not in use.
  - Wash hands before and after the practical work.
  - All cultures should be autoclaved before disposal.
  - It is advisable to wear a face mask when dealing with sporulating fungal cultures.
- Penicillium expansum* does not produce penicillin but anyone who suffers from allergies should be especially careful and must wear a face mask.

**1** Examine the fungal colony on the agar plate

Describe its appearance.

Note

- colour
- appearance
- diameter of colonies



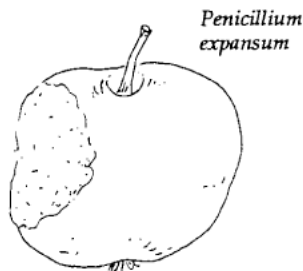
*Penicillium expansum*

**2** Examine the infected apple (externally)  
Compare with the control

Apple 1 has been inoculated with *Penicillium*.  
Apple 2 is the control (see above)

Note

- colour
- shape
- texture
- size of infection



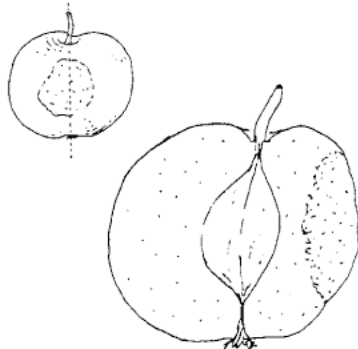
*Penicillium expansum*

**3** Examine the infected apple (internally)  
Compare with the control

Cut the infected and the control apples in half, through the inoculation point. Describe the type of rot you see.

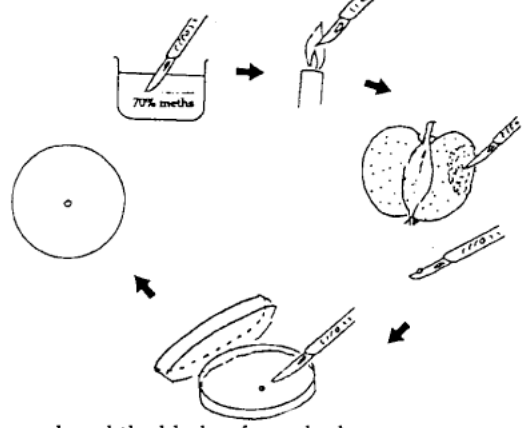
Note

- colour
- texture
- size of infection



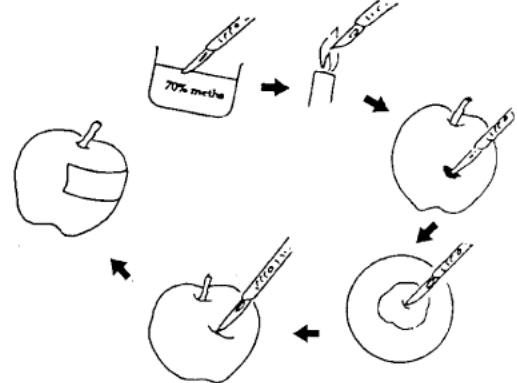
► Record your observations for steps **1**, **2** and **3** in a table.

**4** Isolate the organism from the infected material (apple 1) and transfer to culture on a plate



Flame and cool the blade of a scalpel.  
Cut out a small piece (5 mm x 5 mm) of infected tissue from the cut surface of the infected apple.  
Place this in the centre of a clean malt agar plate.  
Replace lid, seal with tape and label.  
Repeat for apple 2 (control).  
Incubate plates, the right way up, at 25 °C for 7 days.

**5** After 7 days you should have one or more fungal colonies on your plate. Infect a new apple using the culture on the plate (from step **4**)



Flame scalpel (or forceps) and cool.  
Make a wound in a fresh apple (apple 3) and insert a small piece of fungal colony from the agar plate.  
Cover wound with micropore tape. Label.  
Incubate at room temperature for 7 days.

**6** Repeat step 5 using the fungal colony (if any) from the control apple. Examine each day for 7 days.

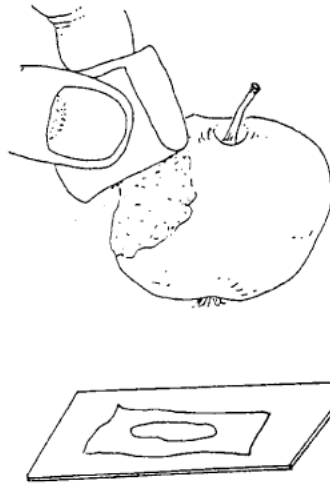
► Record your results in a table as before. Are your observations the same as you recorded earlier?

Now check through to see if you have demonstrated Koch's Postulates (see statements below). If your answers to numbers (iv) and (v) are 'yes', then you have isolated the organism which is causing the disease.

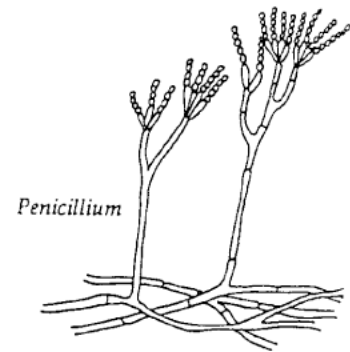
Acknowledgements to: Dr Mary MacDonald (formerly of SAPS) and Sue Hocking (Trowbridge College)

© SAPS This document may be photocopied for educational use in any institution taking part in the SAPS programme. It may not be photocopied for any other purpose.

## Examining the fungus under the microscope



- ① Cut a piece of sellotape and hold both ends in one hand so that the sellotape forms a loop with the sticky side outwards.
- ② Lightly touch the loop of sellotape against the surface of the infected tissue, or onto a fungal colony on a plate.
- ③ Stick the sellotape on the slide. Do not press it in the middle.
- ④ Observe under low power and then under high power.



- ⑤ Note the conidiophores with the conidia.

**Some questions for you to answer** – Fungal spores may be visible on the surface of the apples. In nature, how do you think these spores are dispersed? What features help in their distribution? What protection do apples have to prevent infection (think about the control apple)?

## Media recipes for fungal practicals

Multi-purpose media for fungal isolation and growth. All media autoclaved at 15 p.s.i. 121 °C for 15 mins.

### Malt agar

Malt extract 20 g, agar 20 g, distilled water to 1 dm<sup>3</sup>

You can use malt extract without additives from the chemist, but will need to adjust the pH to 6.5 using NaOH.

Chemicals and powdered media may be bought from:

- Sigma-Aldrich Co Ltd 0800 447788
- Philip Harris Educational 01543 480077
- Merck BDH 0800 223344

Fungal cultures may be purchased from:

- Blades Biological 01342 850242
- Philip Harris Educational 01543 480077

## Notes

Mouldy fruits and vegetables brought in can be used for this practical. These are likely to contain more than one organism, one of which is the causative agent and the others are opportunists, taking advantage of the decaying or dead material. There is, however, some risk with unknown *Penicillium* spp., as some produce penicillin. You are reminded that you should always avoid breathing in spores of unknown fungi and you are therefore advised to wear a face mask.

Other suitable apple fungal pathogens which can be used include *Monilinia fructigena*, *Nectria galigena* and *Botrytis cinerea*. *Monilinia* produces a firmer but more extensive rot. Infection triggers the production of phenolics which are oxidised by host phenolases giving brown compounds which, in turn, inhibit the production of fungal pectolytic enzymes. *Penicillium* produces an inhibitor of the phenolases so there are fewer brown products and the pectolytic enzymes are able to break down pectins in middle lamellae of apple tissue and separate the cells, giving a paler, softer rot.

This worksheet provides practical work which can help teach and illustrate many topics. These include fungal structure and nutrition; cell, tissue and plant structure; enzyme activity; decay, carbon cycle; mechanics of disease transmission, epidemiology; gene switching. From this, there are plenty of ideas for extended investigations. These practicals pose minimum risk to health, and give the opportunity to enforce good laboratory practice and the importance of aseptic technique.

## Useful references

- Ingold C T & Hudson H J (1996) *The Biology of the Fungi*. Chapman & Hall. ISBN 0-412-49040-4
- Margulis L & Schwartz K V (1988) *Five Kingdoms - 2nd edition*. Freeman. ISBN 0-7167-1912-6
- Webster J (1991) *Introduction to Fungi*. Cambridge University Press. ISBN 0-521-29699-4
- Wheeler B E J (1976) *An Introduction to Plant Disease*. John Wiley & Sons Ltd ISBN 471-93751-7
- SAPS web site: <http://www-saps.plantsci.cam.ac.uk>