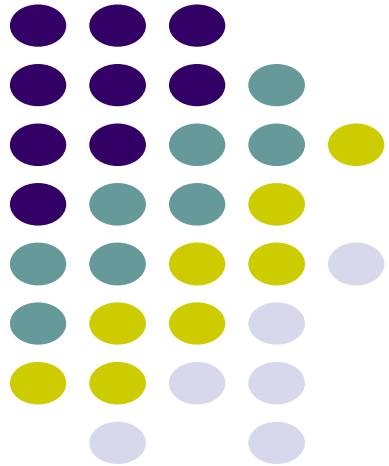
Microscopic techniques and staining methods



Microscopy types

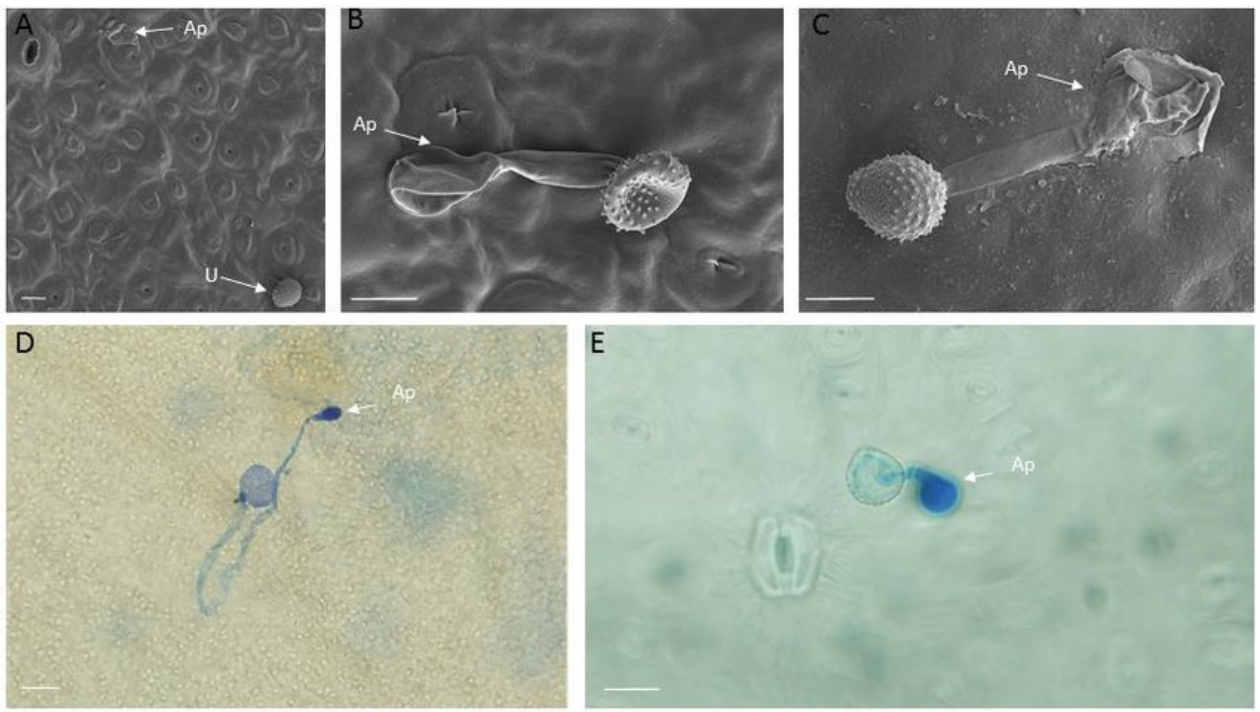
- Optical microscopy
- Electron microscopy

- **Optical microscopy:** Conventional light microscopy, Fluorescence microscopy, confocal microscopy
- Otherwise known as light microscopy, it involves the usage of visible light and one or more lens to produce an enlarged image of an object that is placed in the focal plane of the lens. This can either branch off into transmission, where the beam of light passes through the sample. There are many applications to Optical microscopy such as in microbiology, nanophysics and biotechnology but in plant pathology it is mostly known for using in diagnosis of infected plant tissues. Light microscopes normally have three types of magnifications
  - Low power (10X)
  - High power (40X)
  - Oil immersion (100X)

Normal Binocular Microscope

Inverted Binocular Microscope

- **Electron microscopy:** Scanning electron microscopy (SEM), Transmission electron microscopy (TEM)
- This is a form of microscopy that uses electron beams to create an image of the object being used. They have a much higher magnification than light microscopes. This allows us to see smaller specimens in greater detail. The resolution is able to be increased because as the electrons travel faster their wavelength becomes shorter so there is a direct correlation between reducing wavelength and increasing resolution.
- There are 2 types of electron microscopes used, Transmission and Scanning electron microscopes. TEM involves shooting a high voltage beam through a thin layer of specimen and gathering information about the structure. SEM in contrast produces images by detecting secondary electrons that have been emitted off the surface due to excitation by the primary electron beam.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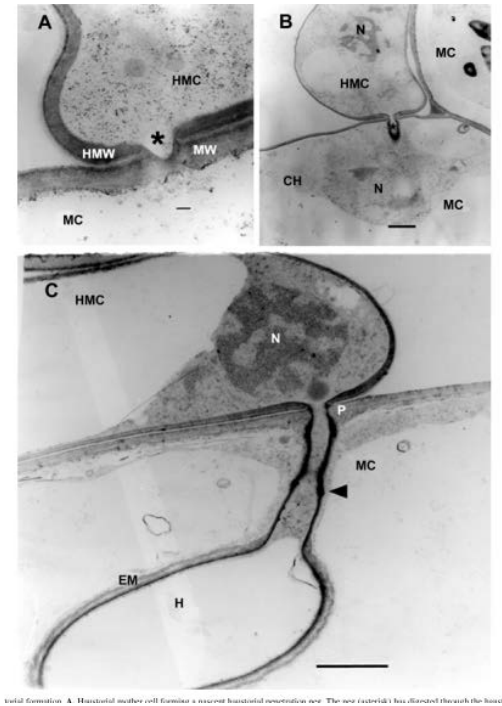
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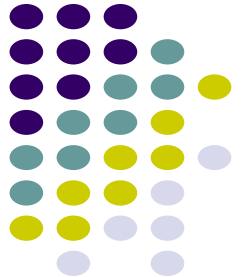
Staining of fungal structures



- **Lactophenol Cotton Blue Stain**
- It is formulated with lactophenol, which serves as a mounting fluid, and **cotton blue**. Organisms suspended in the **stain** are killed due to the presence of phenol. **Cotton blue** is an acid dye that **stains** the chitin present in the cell walls of fungi.
- **Phenol:** kills any live organisms;
- **Lactic acid:** It preserves fungal structures, and
- **Cotton blue:** It stains the **chitin** in the fungal cell walls.

Preparation of lactophenol cotton blue (LPCB)

- **Ingredients**
  - Cotton Blue (Aniline Blue) 0.05 g
  - Phenol Crystals ($C_6H_5O_4$) 20 g
  - Glycerol 40 mL
  - Lactic acid ($CH_3CHOH\ COOH$) 20 mL
  - Distilled water 20 mL
- **Method**

This stain is prepared over two days.

- On the first day, dissolve the Cotton Blue in the distilled water. Leave overnight to eliminate insoluble dye.
- On the second day, wearing gloves add the phenol crystals to the lactic acid in a glass beaker, place on magnetic stirrer until the phenol is dissolved.
- Add the glycerol.
- Filter the Cotton Blue and distilled water solution into the phenol/glycerol/lactic acid solution. Mix and store at room temperature.

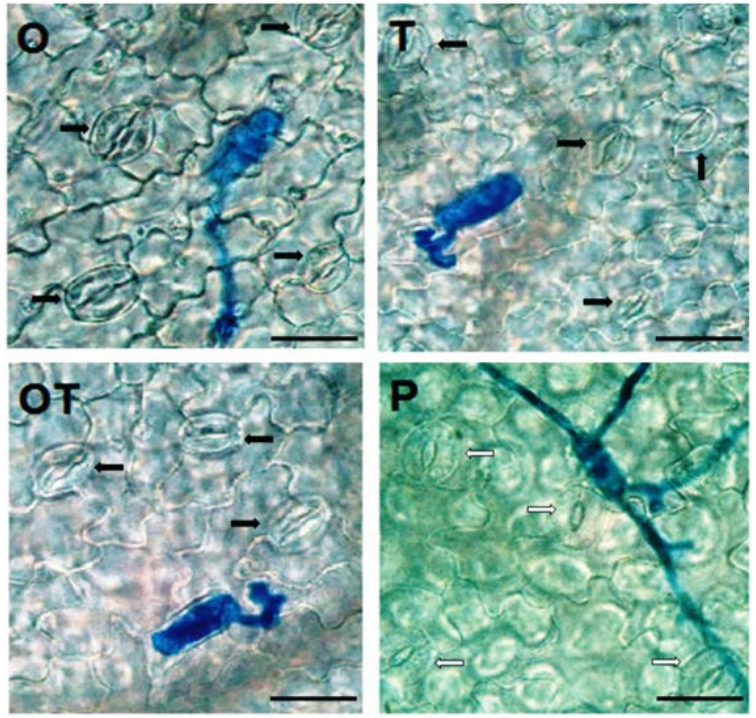
Observing fungal spores on glass slides

- Hyaline spores (High power 40X)

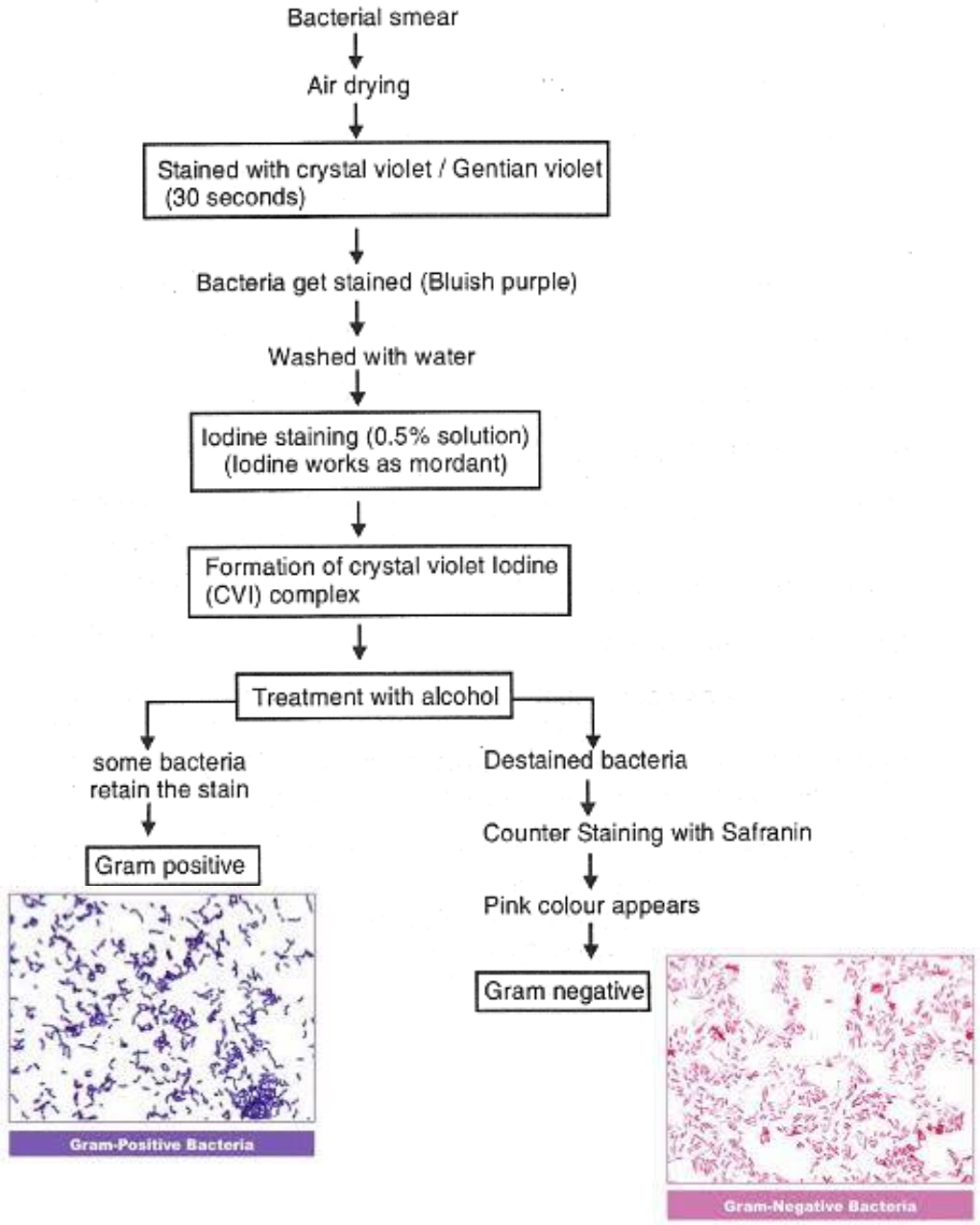

- Coloured spores (High power 40X)

Observing fungal spores on leaf surface

- **Leaf clearing technique**
- Ethanol, acetic acid solution in the ratio of 3:1 is used for removal of chlorophyll from the leaves. After clearing the leaves, staining was done with Coomassie blue (0.01% in methanol) for visualization of *Erysiphe pisi* conidia on leaves. After staining, leaves are kept on glass slide and observed under compound light microscope for conidial presence and their germination.



Gram staining of bacteria



Observe under oil immersion (100X) after covering with cover slip