

## **HISTOPATHOLOGY OF BACTERIAL INFECTION**

The presence of bacterial pathogens in plant host tissues and the change they cause in these plant tissues are studied by histopathology of infected plant material. The location of the bacterial pathogen as intercellular or intracellular or in water conducting tissues can be revealed with microtome sectioning and staining of the plant cellular material.

### **DETERMINATION OF THE LOCATION OF BACTERIAL PATHOGENS IN HOST**

To determine the exact location of bacteria in diseased tissue, it is desirable that the material is fixed in a suitable killing and fixing solution. In sections of fresh material, considerable bacterial population may be lost when sections are transferred to water from the razor. The most desirable killing and fixing solutions are formalin–acetic acid–alcohol (FAA) and the FAA saturated with mercuric chloride. The advantage of the latter is that it helps to bind the bacterial cells together and thus keeps the bacterial mass intact without leaching or dispersal during sectioning and staining. Even the bacterial exudates on the surface of the tissue are preserved in this fixing solution.

**Composition** of Fixing Solution (FAA) Alcohol (50 percent) .... 100 ml Formalin .... 6.5 ml Acetic acid .... 2.5 ml

**Material Required** Disease sample, microtome, razorblade, fixing solution, distilled sterile water glass slide coverslip, tissue paper, differential stains, alcohol, xylol, balsam, microscope, and so on.

**Procedure 1.** Fixing the material: Wash the diseased sample in running tap water to remove dirt and external microbes. Dry with blotter paper/tissue paper. Cut the material into 5 mm pieces with microtome and transfer in FAA. The minimum time required for killing and fixing is 48 hours, but the material can be kept for any length of time. In FAA saturated with mercuric chloride, the tissue pieces are kept for 48 hours, washed in FAA to remove excess mercuric chloride, and then preserved in FAA. 2.

**A. Staining of free hand sections:** The sections can be cut with a sharp razoror news having blade, and stained by the following methods. a. Staining with acid fuchsin: Mount the sections in 0.1 percent acid fuchsin in lacto phenol; allow to stand for 5 to 10 minutes and examine. Observation The bacteria are stained deep red and the host cell walls pink. In the affected tissues of coleoptiles,

leaf sheet, stem, and so on, which contain little or no chlorophyll, the bacterial cells can be revealed prominently by this method.

**B. Staining with differential stain:** Stain the section with carbol thionin for 5 minutes (Thionin blue 0.1 gm, phenol crystals 5.0 gm, water 100 ml). Wash in water and transfer to 95 percent alcohol for a minute. Put in orange G for a few minutes (orange G saturated in absolute alcohol). Wash in absolute alcohol. Clear in xylol and mount in balsam.

**Observation** The bacteria stain deep blue, the cellulose cell walls yellow or green, and the liquefied tissue light blue.