Chapter - 10

Identification of Fungi

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- The most significant fungus characteristics used for identification are spores and spore-bearing structures (sporophores) and, to some extent, the characteristics of the fungus body (mycelium).

- These items are examined under a compound microscope directly after removal from the specimen. The specimen is often kept moist for a few days to promote spore development. Alternatively, the fungus may be isolated and grown on artificial media and identified on the basis of spores produced on the media. For some fungi, special nutrient media have been developed that allow selective growth only of the particular fungus, allowing quick identification of the fungus.

- The shape, size, color, and manner of arrangement of spores on the sporophores or in the fruiting bodies, as well as the shape and color of the sporophores or fruiting bodies, are sufficient characteristics to suggest, to one somewhat experienced in the taxonomy of fungi, the class, order, family, and genus to which the particular fungus belongs.

- In any case, these characteristics can be utilized to trace the fungus through published analytical, often dichotomous keys of the fungi to the genus and, finally, to the species to which it belongs.

- Once the genus of the fungus has been determined, descriptions of the known species are found in monographs of genera or in specific publications in research journals. Because there are usually lists of the pathogens affecting a particular host plant, one may use such host indexes as short cuts in quickly finding names of fungus species that might apply to the fungus at hand.

- Host indexes, however, merely offer suggestions in determining identities, which must ultimately be determined by reference to monographs and other more specific publications.

- In many fungi, hyphae in a colony or in adjacent colonies fuse

(hyphal anastomosis). If the hyphae that fuse carry genetically different nuclei, the colony that is produced is a heterocaryon.

- Many fungi, however, have genetic systems that prevent mating between genetically identical cells. If the hyphae that come in contact belong to different strains of the same species but are of the same mating type, their encounter may result in vegetative incompatibility. Thus, the resulting vegetative incompatibility between colonies of various strains belonging to the same species is used to type the strains as belonging to different incompatibility groups constituting different biological species.

- In recent years, immunoassay techniques, often involving monoclonal antibodies against specific proteins of a fungus conjugated with a fluorescent compound, have been used for the detection and identification of certain fungi.

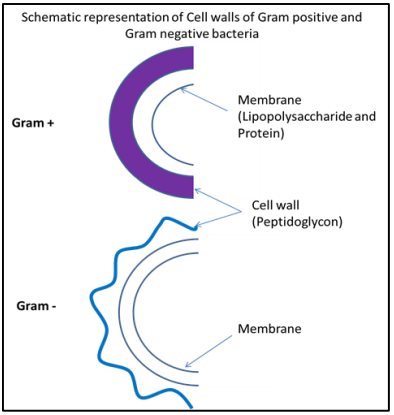
- The advent of molecular techniques, particularly of the polymerase chain reaction (PCR), of quick and inexpensive sequencing of DNA, and the accumulation of a relatively large databank of ribosomal DNA sequences have revolutionized both the lower limits of detection of pathogens and the accuracy and rapidity of their identification. These developments have made possible the detection of pathogens within plant tissues in the early stages of infection while there is still a minimal presence of the pathogen and early intervention may prevent an epidemic.

- They have also made possible a definitive identification of the pathogen by using DNA probes of known pathogens and, furthermore, they have made possible the quantification of the pathogen within, or in a mixture with, plant tissue, such as seed. Most DNA primers are for internal transcribed sequences of ribosomal DNA. The methodology, however, improves constantly and quickly.

- Much more sensitive and specific sets of primers have been developed based on families of highly repeated DNA that were 10 times more sensitive than primers directed at internal transcribed spacer sequences for ribosomal DNA.

Identification of Bacteria

The simple staining procedure makes to visualize bacteria clearly, but it does not distinguish between organisms of similar morphology. In 1884, a Danish Physician named, **Christian Gram** discovered a new technique to differentiate the bacteria of similar morphology. One of the most important cytological features of bacteria is their reaction to a simple procedure called after its discoverer, the Gram stain.



Hans Christian Gram

The peptidoglycans of Gram-positive bacteria are more extensively cross-linked than those of Gram-nagative. The chemical compositions of certain substances in bacterial cells can be detected with specific staining techniques. Information about the presence or absence of such substances is used for the identification of bacteria.

Gram's staining reaction differentiates bacteria into gram-positive and gram-negative types.

In this reaction, bacteria fixed on a glass slide are treated with a crystal violet solution for 30 seconds, rinsed gently, treated with iodine solution, and rinsed again with water and then alcohol.

Gram-positive bacteria retain the violet-iodine stain combination because it forms a complex with certain components of their cell wall and cytoplasm.

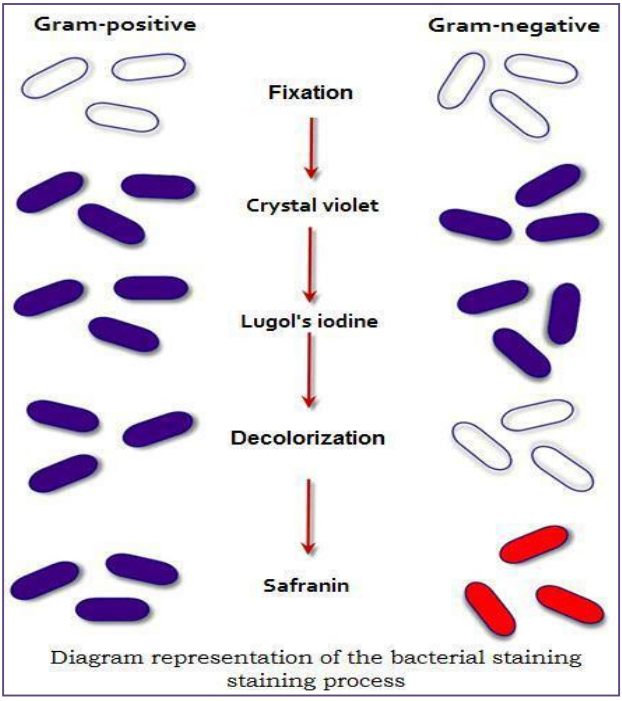
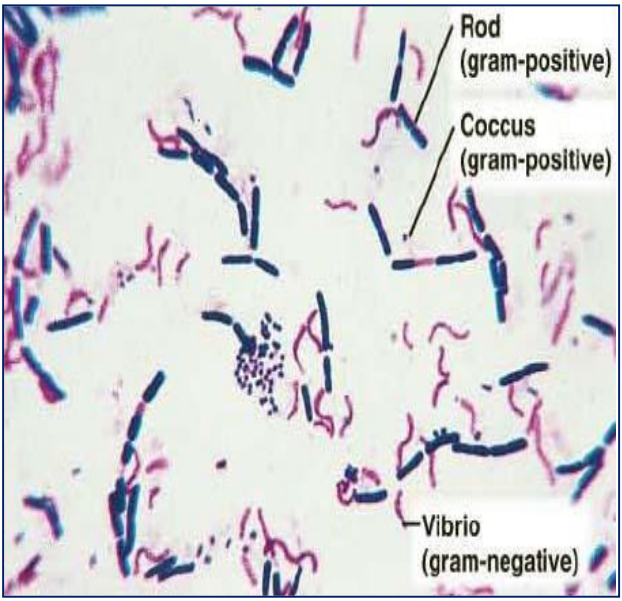


Diagram representation of the bacterial staining staining process



Gram-negative bacteria have no affinity for the stain combination, which is therefore removed by the alcohol rinse, and bacteria remain as

nearly invisible as before. Of the rod-shaped phytopathogenic bacteria, only the genera *Clavibacter*and *Curtobacterium*, and the relatively unimportant plant pathogens of the genera *Arthrobacter*, *Bacillus*, and *Rhodococcus*, are gram positive. *Agrobacterium*, *Erwinia*, *Pseudomonas*, *Ralstonia*, *Xanthomonas*, and *Xylella*are gram negative.

Gram positive bacteria	**Gram negative bacteria**
1. Cell wall thicker, contains only traces of lipids; 2. Striking simplicity of amino acids, glutamic acid occur in large amounts; 3. Diaminopimelic acid in some; 4. More muramic acid; 5. Teichoic acid present.	1. Cell wall thinner, may contain upto 20% lipids; 2. All the amino acids are present; 3. Daminopimelic acid in some; 4. Less muramic acid; 5. Teichoic acid absent.

Pathogenicity Test

Phytopathogenic bacteria are also tested for their pathogenicity on various species and varieties of host plants. This test, for practical purposes, may be sufficient for tentative identification of the bacterium. In many cases, the effort to establish the identity of an isolated bacterium begins with observation of the external symptom, e.g., a plant appears wilted or the spots on the leaves are surrounded by a halo [Fig.1].

The next step is observation of some of the easier internal symptoms, e.g., the wilted plant shows discoloration of the vascular system, so the wilt is caused by a pathogen and not by drought [Fig.2].

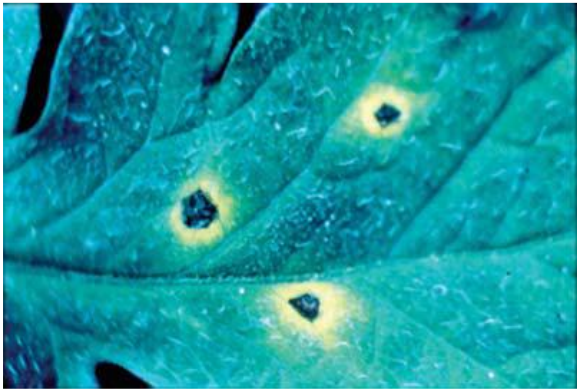


Fig.1

Fig.2

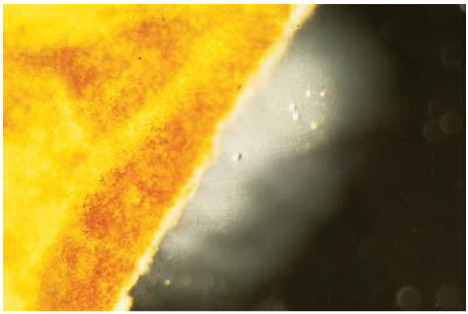


Fig. 3

Further examination of the wilted plant can be done by placing a freshly cut wilted stem in a tube or dish of water and looking for appearance or lack of a cloudy diffusatefrom the stem, which, if present, indicates that the wilt is cause by bacteria rather than a fungus or anything else [Fig.3].

Fig. 4

Fig. 5

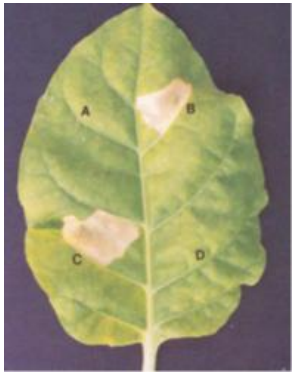
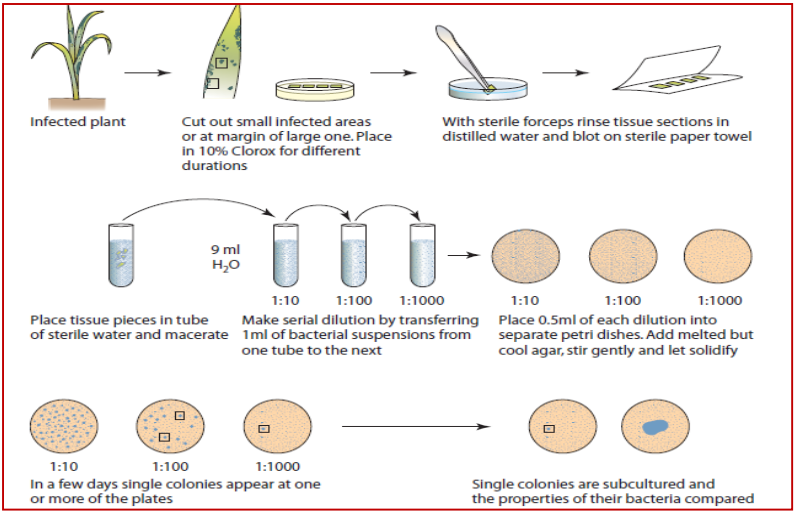


Fig. 6

By being familiar or comparing the literature about which bacterium causes symptoms like that observed in this particular host, one can identify the bacterium and diagnose the disease. If further work is needed, then one cultures the bacteria and observes the shape, size, color, and so on of its culture [Fig.4].To make sure that the isolated bacterium is the pathogen rather than saprophyte, a series of dilutions of the bacteria is injected into the leaves of a nonhost, such as tobacco. If nonpathogenic, the leaves show no change at the points of injection [Fig.5].

If the bacterium is pathogenic, it produces a **hypersensitive response** (dead tissues around the points of injection) [Fig.6].



Isolation of bacterial pathogens from infected plant tissue

An excellent method of isolation and identification of bacteria obtained from plant tissues or soil is through the use of **selective nutrient media**. Selective media contain nutrients that promote the growth of a particular type of bacterium while at the same time contain substances that inhibit the growth of other types of bacteria. Positive identification usually requires more than one sub culturing on selective media because seldom does only one bacterium grow on a selective medium. The available selective media for plant pathogenic bacteria are helpful for routine isolation and sometimes identification of bacterial genera and of several species and even pathovars.

Diagnosis of Plant Viruses

Because different viruses may elicit similar symptoms, the disease phenotype can provide only limited, although important information for disease diagnosis. More specific and reliable methods of virus identification are based on various properties of the virus. These approaches include:

1. Pathogenicity – Bioassays using indicator plants. Some plant genera, such as Nicotiana (tobacco) and Chenopodium (lambsquarters) are hosts for a number of viruses. These plants have consistent and distinctive responses hence they are commonly used as indicator plants. Local lesions and systemic infections are the main responses elicited. Many plant viruses are transmissible to indicator plants by means of mechanical transmission or grafting.

2. Transmissibility – Vector transmission assays. Because of vector

specificity, identification of the organism that transmits the virus may provide important information for virus identification.

3. Architecture of virus particles – this makes use of electron microscopy and it utilizes the shape and size of virion to distinguish rod-shaped, filamentous, icosahedral, or large enveloped particles.

4. Presence of virus-specific structures in infected cells – following their association with components of the cell, viruses often form unusual structures within plant cells as a result of infection. Viruses in the family Potyviridaeproduce 'pinwheel' inclusions which are not found in healthy cells or cells infected with other viruses. The detection of these virus-specific inclusions indicates the presence of a virus within that group.

5. Properties of the protein coat –this makes use of immunological procedures. These tests rely on identification of a virus (the antigen) through its reaction with specific antibodies. Specific antibodies are produced by an animal when foreign proteins are introduced into the animal. One of the most widely used diagnostic tests for plant viruses is an antibody-based procedure called the Enzyme-Linked Immunosorbent Assay (ELISA).

6. Properties of viral nucleic acid – this involves PCR amplification which is a very sensitive and specific technique for virus detection that is based on the presence of unique nucleic acid sequences in the genome of a virus. The viral DNA sequence that a researcher wants to amplify undergoes about thirty cycles of copying in a small test tube.