FUNGAL DISEASE IDENTIFICATION

BSc I Botany Subs.

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Fungal diseases identification

For a correct identification of the causal agent of plant diseases and make reliable conclusions, appropriate isolation procedure must be followed. The successful isolation of fungi from diseased plants depends on several factors:

- Type of diseased tissue (seeds, leaves, stems, roots)
- Method of surface sterilization
- Plating procedure
- Isolation medium
- Incubation conditions

A. Isolation from leaves and stems

Avoid using potato dextrose agar (PDA) or other carbohydrate-rich media for isolation from diseased plant tissues, especially if isolating from roots. Saprophytic fungi and bacteria grow quickly on carbohydrate-rich media and suppress the growth of slower growing fungal pathogens.

Isolation from stems is often improved by removing the bark or outer stem tissues before surface sterilization.

1. Wipe the work area with 70% ethyl alcohol.

2. Dip instruments (forceps and knife or scalpel) in 70% ethyl alcohol and flame dry.

3. Rinse leaf or stem tissue in water to remove soil and other debris.

4. Surface sterilize leaf or stem tissue by wiping the surface with soft paper (paper tissue) dipped in 70% ethyl alcohol or by briefly dipping thick leaves in 70% ethyl alcohol for 5 seconds, rinsing in sterile water and damp-drying on sterile paper tissue.

5. Aseptically cut small pieces (approximately 2 × 2 mm) from the margin of the healthy and diseased tissue, and transfer them to a low-nutrient medium (e.g. water agar [WA]) or a selective isolation medium, placing the pieces near the side of the plate.

6. Incubate the plates at approximately 25°C, ideally under lights.

7. Check plates each day, and when fungal colonies develop from the pieces of plant tissue, transfer material from the margins to a medium such as PDA or WA that contains sterile pieces of plant tissue, for example, pieces of green rice stem, carnation leaf or bean pod. 19 (Sterile pieces of plant tissue encourage sporulation, which aids in identification of the pathogen.)

8. Make a final identification using pure cultures grown from a single germinated spore or a hyphal tip.

B. Alternative method for isolating from leaf spots

1. Place the leaf or leaf piece on moist paper in a Petri dish in a humid chamber.
2. Incubate at approximately 25 °C under lights to promote sporulation.
3. Examine after 1–2 days under the dissecting microscope to locate spores or sporeforming structures such as pycnidia, acervuli or sporodochia.
4. Pour isolation plates containing WA with a drop of lactic acid (which reduces the pH and suppresses bacterial growth) or with added antibiotics.
5. Using a sterile transfer needle, transfer the spores to the plates.

C. Isolation from seeds

1. Soak seeds in 1% NaOCl for 1-5 minutes (based on the texture of the seed coat)
2. Soak seeds for 2 min in 70% ethanol and rinse in 3 changes of sterile distilled water.
3. Dry the seeds on sterile paper tissues before plating on moistened blotter or agar.

The blotter technique

1. Place sterile blotting paper (3-layers) in sterilized Petri dishes.

2. Moisten the papers with sterilized distilled water so that little amount of surplus water will be left on the surface of the papers

3. Place surface disinfested seeds on the water soaked blotters and keep them in a controlled room at a temperature of 20±2ºC under a pair of tube light mounted at about 40cm above the seeds with alternating cycles of 12hr near ultraviolet light and darkness.

4. Examine the growing fungi on the seed after 8days of inoculation and record the data.

D. Isolation from small, thin roots

Do not use severe surface sterilization of small rootlets as the sterilant may kill all the fungi in the rootlet, including the pathogen.

1. Select diseased rootlets with both healthy (symptomless) and diseased parts, and wash them in three changes of sterile water in a small bottle. Add a small drop of detergent to the first wash.

2. Wipe the work area with 70% ethyl alcohol.

3. Dip instruments (forceps and knife or scalpel) in 70% ethyl alcohol and flame dry.

4. Dip the rootlets briefly in 70% ethyl alcohol, rinse quickly in sterile water and then damp-dry on sterile paper tissue. Alternatively, surface sterilize the rootlets in 1% sodium hypochlorite or in 10% ethyl alcohol for 10–15 seconds only, immediately rinse in sterile water and allow to air-dry on sterile paper tissue in a sterile work chamber.

5. Aseptically cut root pieces 1–2 mm in size at the margin of healthy and diseased tissue and transfer onto WA or a selective medium.

6. Press the pieces gently into the surface of the agar to ensure good contact between the entire root segment and the antibiotics in the agar.

7. Incubate at approximately 25 °C and check each day under the dissecting microscope for fungal growth from the root pieces.

8. Subculture each colony onto PDA or WA containing sterile pieces of plant tissue, such as green rice stem pieces.

9. Purify by hyphal tipping or by the single germinated spore technique before final identification.

Final Identification of fungal pathogens

- Microscopic examination is generally required to enable identification of the pathogen and diagnosis of the disease. The identification of fungal pathogens is based initially on morphological features, such as spores and spore-forming structures. For example, most fungal pathogens that cause leaf diseases produce spore forming structures; perithecia, pycnidia, acervuli, sporangiophores or conidiophores that can be readily examined microscopically and, to some extent, the characteristics of the fungus body (mycelium).
- 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.
- 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.
- Books, keys and manuals should be kept as resources in diagnostic laboratories, and fortunately many scientific journal publications on taxonomy and identification can be accessed via the internet.