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Study on Isolation of Microorganism

Isolation: Isolation means separation of a plant pathogen from its host or source and growing in pure culture on an artificial medium.

Objectives:
1. To establish pure culture of an organism
2. To study the different properties of microorganism
3. To identify the microorganism
4. To perform the various phytopathological and microbiological studies.

Method of isolation:

Sl	Methods	Pathogens involved
1.	Tissue Plating Method	for many leaf-colonizing fungi
2.	Direct Observational Method	for fungi that sporulate readily
3.	Moist Chamber	isolation from incubated substrates in a Moist Chamber
4.	Particle Filtration Technique	for fungi on wood and plant debris
5.	Induction of mycelial growth	for deep seated infections and poorly sporulating fungi
6.	Dilution technique	for bacteria & fungi with large number of reproductive bodies
7.	Suspension Plating	For soil bacteria and fungi that grow easily
8.	Direct isolation/Baiting method	Organisms that are present in pure colonies
9.	Host inoculation	badly contaminated and only for obligate parasite such as Virus
10.	Dissection	for nematode
11.	Baermann funnel	for nematode

Preparation for isolation
1. Glassware, such as petri dishes, test tubes and pipettes etc. must be sterilized.
2. Surface sterilants solutions should be prepared for treating the surface of the infected or infested tissue to eliminate or markedly reduce surface contaminants that could interfere with the isolation of the pathogen. The most commonly used surface sterilants are 0.5 percent sodium hypochlorite solution (3-5 minutes), used both for wiping infected tissues or dipping sections of such tissues in it and 70 percent ethyl alcohol (3-10 seconds) which is used for washing/dipping leaves approximately three seconds or more. The tissues must be blotted dry with a sterile paper towel.
3. Culture media to be prepared according to the isolated fungal or bacterial pathogen which will be grown. Fungi can also be separated in culture from bacteria by adding 1 or 2 drops of a 25% solution of lactic acid to 10 milliliters of the medium before pouring it into the plate which inhibits the growth of bacteria.

Isolation of fungi from soil

Procedure:

Simple plating technique (Direct isolation):

1- Transfer a small amount (5-15 mg) of soil to a sterilized petri dish

2- Add 8–10 ml. of semi-cooled (45°C) nutrient medium and shake the plate to let the soil particles disperse throughout the thin layer of agar medium before it solidify

3- If the soil is very dry, or contains a high proportion of clay, it is preferable to mix the particles with a drop of sterile water in the plate before adding the medium

4- Incubate treated plates at 25-30°C, investigate the mycelia appearance after 5-7 days and record the results.

Dilution (Plate) Method:

1. Take a proper amount of airy dried soil sample after removing any undesirable materials (plant debris and big granules)
2. Prepare serial dilution (i.e. 1:10, 1:100, 1:1000…….etc.) from the soil sample
3. Transfer one drop from each of the last two dilution samples to culture media using a sterile pipette
4. Use golf shaped like glass road to spread the droplets onto the agar surface
5. Incubate treated plates at 25-30°C, investigate the mycelia appearance after 5-7 days and record the results.

Isolation of fungi from diseased plant parts

Procedure:

Tissue Plating Method:

1. Use running tap water to wash plant materials for at least 5-8 min.
2. Select the most freshly infected plant parts (from diseased plants), surface sterilize the selected parts using 0.5% sodium hypochlorite solution or 70% ethanol for 3-5 min or 3-10 seconds respectively. Then, again use sterilize distilled water to wash plant materials for at least 1-2 min.
3. Cut each surface sterilized plant part into tissue segments of less than 5 mm.
4. Transfer prepared tissue segments to culture media using sterilized forceps (3-4 segments/ 9 cm plate)
5. Incubate cultured plates at the appropriate temperature (25-30°C) for 5 to 7 days
6. Investigate the incubated plates regularly because fungal hyphae may be elongated from the plated tissue segments within a few days. Finally, transfer any appeared hyphal tip to a new culture plate as soon as possible to avoid any contamination

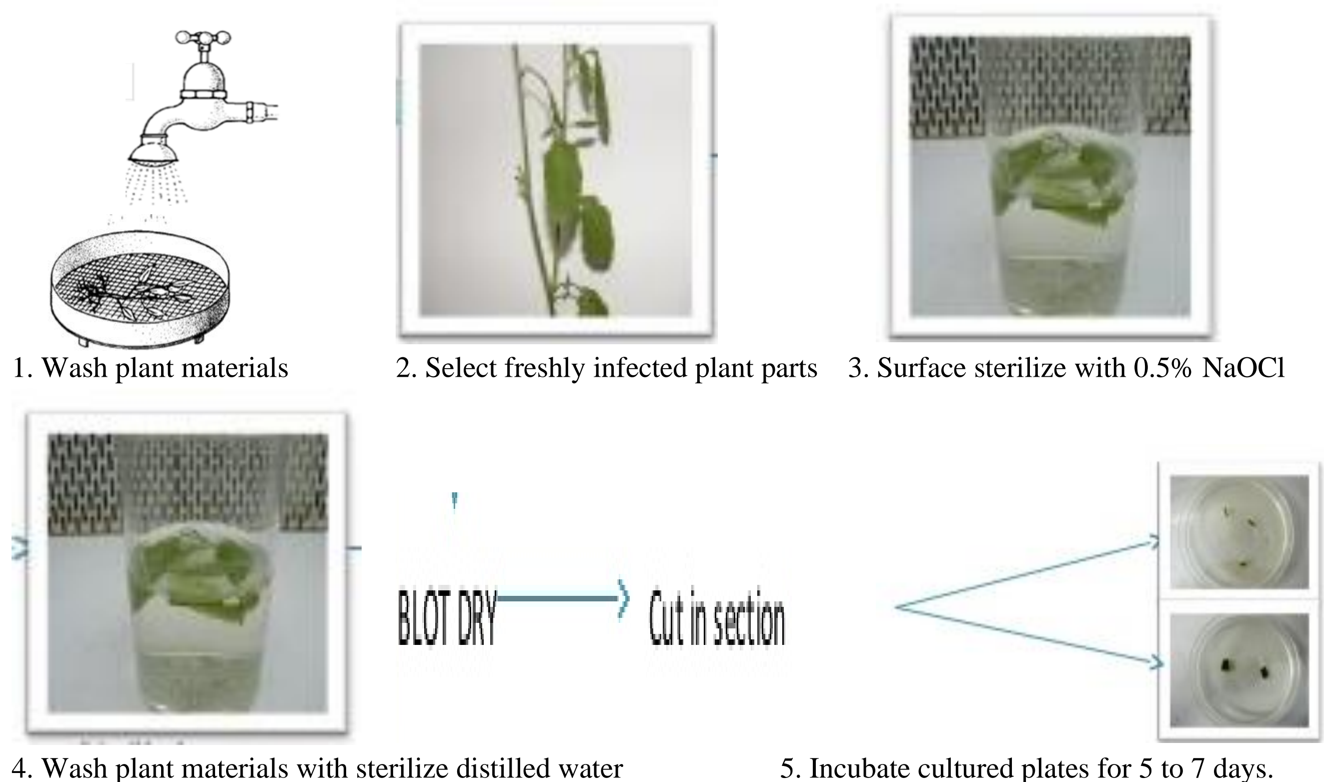

Figure 1. Schematic representation of Isolation of fungi from diseased plant parts

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Isolation of bacteria from plant tissue

Procedure:

Tissue Plating Method:

1. Select a piece of symptomatic tissue
2. Cut along into small pieces- not much tissue is needed
3. Add a small amount of water and grind tissue and mix properly
4. Using inoculating wire loop followed by aseptic technique for streaking the culture plate
5. After that, incubate the culture plates at 25-30°C for 2-3 days
6. Observe the plates for growth and choose individual colonies- lightly touch the top of an individual colony with a sterile loop or needle and streak on a fresh agar plate for getting pure culture.

Isolation of soil bacteria

Procedure:

Dilution (Plate) Method:

1- Suspend 1 grams of air-dried powdered soil in 10 ml sterilized distilled water and shaken for 15 minutes.
2- Make serial dilutions of previous stock in glass test tubes.
3- Use the last two dilutions and take one wire loop of the soil dilution to inoculate culture plate
4- After that, incubate the culture plates at 25-30°C for 2-3 days.

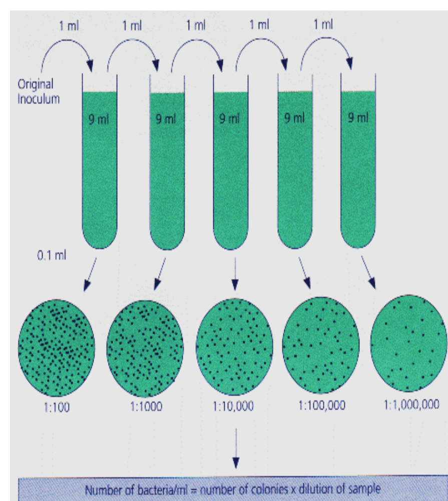

Figure 2. Serial dilution method

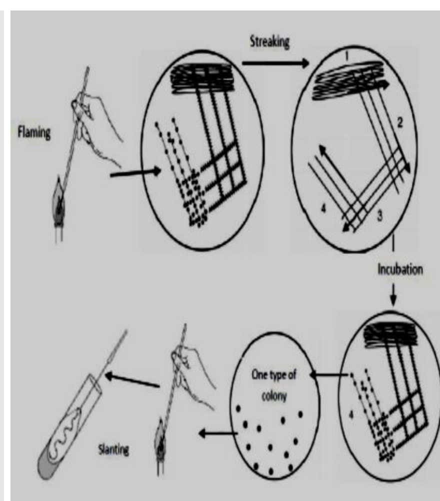

Figure 3. Streak Plate Method

Isolation of Nematode

Extraction from plant material by dissection method:

The method is suitable for diagnostic purpose. Infected plant material is analyzed under a microscope for the presence of nematodes.

Procedure: Carefully wash the plant material and place it in a water-filled petri dish. Dissect the sample with dissecting needles and forceps under dissecting microscope. The emerging nematodes, egg masses, etc. will be picked from the suspension with a handling needle or painting brush. After 2-3 hours, the sample should be observed again, because active stages of nematodes may have crawled out of the material.

Extraction of active nematodes from plant material/ soil by Baermann funnel method:

The Baermann funnel is used for extraction of active nematodes from plant material and soil. The sample size depends on the funnel diameter and the type of material. This method makes use of nematode mobility. When plant material/soil is placed in water, nematodes crawl out of the material and sink.

Procedure:

1. Set up a ring stand. Attach hose to funnel and place funnel with hose into ring of ring stand. Secure clamp to hose attached to funnel. Place circular piece of wire screen inside of funnel.
2. Add tap water to the funnel until the water surface is barely touching the wire supporting screen. At this time, make sure that water is not leaking from the tube.
3. Place an open sheet of two-ply facial tissue over the supporting screen in the Baermann funnel, letting the edges of tissue drape over the outside edge of the funnel.
4. Carefully add approximately ½ to 1 cup of freshly collected soil onto the open facial tissue. Gently spread the soil out in an even layer.
5. Fold over all four corners of the open tissue onto the soil placed on the tissue.
6. Carefully add additional water to the funnel until the water surface is almost above the top of the tissue.
7. Let the Baermann funnel sit undisturbed for 24 to 48 hrs at room temperature. Add additional water to the funnel periodically (once a day should be plenty).
8. Then, carefully release the clamp on the hose attached to the funnel, and collect 5 to 10 ml of solution in a petri plate or watch glass for observing nematodes.
9. Observe solution with a dissecting microscope for the presence of plant-parasitic and free-living nematodes. It can be easily differentiate between stylet-bearing (most likely plant-parasitic) and non-stylet-bearing (free-living) nematodes at the highest magnification of the dissecting microscope.

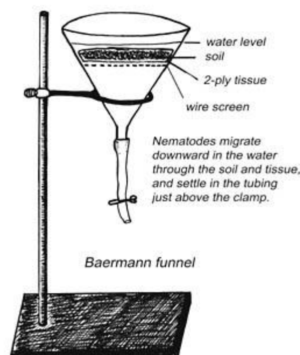

Figure 4. Extraction of active nematodes from plant material/ soil by Baermann funnel method

Plant Virus Isolation and Purification

Virus isolation and purification is a complex process. Depending on the virus and host, it can be achieved in short period or sometimes it would take extremely long periods. Several factors can influence the ease with which virus isolation and purification can be achieved. Stable viruses that reach high concentration in host plants are easy to purify. Each and every virus and host system needs unique procedure to achieve optimum results.

Detection:

Several factors influence the method to be used for virus detection. These include-

- Facilities and expertise available
- Type of virus suspected to be present
- Host plant
- Time available

Enzyme-linked Immuno Sorbent Assay (ELISA) and polymerase Chain Reaction (PCR) are the most widely used virus detection methods because of their rapidness and sensitivity.