Aim

Isolate the fungi from the given soil sample, wet mount and identify the fungi.

Principle

The isolation and identification of soil fungi is an example in which a mycological ecosystem (a particular habitat with its interacting, associated community both abiotic and biotic) can be studied. It is usually too difficult to separate fungi from soil by directly picking them to place under a microscope for observation. Therefore, various techniques have been devised for stimulating growth and consequently easing fungal isolation and subsequently examination.

Techniques used for isolation of fungi from soil include serial dilution agar plate, Warcup soil plate, syringe inoculation, immersion tube method, screened immersion plates, plate profile, hyphal isolation, soil washing, partial presterlization, soil sieving, floatation, baiting etc. A single method cannot be used to count all the different types of fungi present in a given sample. So to have a complete spectrum of fungi present, a sample is processed by a variety of techniques. The dilution plates and soil plate methods are two most widely used methods for fungal isolation from soil.

For rapid and routine examination of almost all types of fungi, spores and spore bearing structures are tested out on slide in a drop of mounting fluid (lactophenol cotton blue) and a cover-glass placed over the preparation which is then ready for microscopic examination.

Requirements

- Soil sample
- Potato dextrose agar media
- Sterile Petri-plates
- Sterile test-tubes
- Lactophenol cotton blue
- Sterile slides, coverslipes
- Sterile Spearder, needle
- Bunsen burner
- Micropipettes

Procedure

1. Collect the soil sample.
2. Prepare the serial dilution (10^{-1} to 10^{-7}) of the soil sample.
3. Transfer 1ml suspension to respective labelled Petri plates using respective pipettes.
4. Pour molten, cooled (45ºC) PDA medium into the Petri plates and rotate the plate gently to ensure uniform distribution of cells in the medium.
5. Allow the medium to solidify.
6. Incubate the inoculated plates for 24-48 hours at 37C in an inverted position and observed it.
7. Preparation of the lactophenol cotton blue microscopic mount –
    a. Place a drop of lactophenol cotton blue on a clean slide.
    b. Transfer a small tuft of the fungus, preferably with spore and spore bearing structures, into the drop, using a flamed, cooled needle.
    c. Mix gently the stain with the mold structures.
    d. Place a cover-glass over the preparation taking care to avoid trapping air bubbles in the strain.
8. Examine the preparation under the low and high power objectives of the microscope.

Observations and results

Observe fungal growth on the plate culture and stain preparation for structure of hyphae and details of sporulating structures microscopically under low and high power. The comman fungi encounterd in tropical soil will include: *Mucor, Rhizopus, Aspergillus, Penicillium, Fusarium, Cladosporium, Alternaria, Curvularia.*

Precautions

1. Soil should be in powered form.
2. Use a fresh sterile pipette in each dilution.
3. Each dilution must be thoroughly shaken before removing an aliquot for subsequent dilution.
4. The air bubble may also be removed by placing the slide in a vacuum chamber.
5. Plates are to be incubated in an inverted position.