Lab No. 3, 4

Isolation & Identification of Fungi

Objectives

- Be able to isolate of fungi from natural sources.
- Be able to identify of fungi strains.

I. Introduction

Fungi can be isolated from virtually any place on earth, from the tropics to the poles and from the mountain tops to the deep oceans. There are many kinds of isolation sources and in our lab we will focusing on: air- borne fungi, soil fungi, infected plant and water fungi.

I.1 Air- Borne Fungi: As we know fungi are present in air in spore shape but sometimes we find hyphae in air.

The mainly function of spores is conserve of species and distribution.

- Factors affecting on the distribution of fungi
  - Temperature.
  - Humidity.
  - Speed of air.
  - Rains.
  - Cycle of spores production.

- Method of exposing the Petri dish: is the simplest method for obtaining of spores from air is to expose Petri dish with nutrient media to air for a period of time then incubate the plate at room temperature.

I.2 Soil Fungi:

- Soil collection: there are many techniques for studying soil fungi and the chose of method are based on the aim of studying. To collect the sample you must take 10- 15 cm of soil approximately 50- 100 gm by clean trowel then store the sample in clean bag.

I.2.1 Studying of soil fungi

- Direct method.
- Indirect method.

I.2.1.1 Direct method: As a result of non- transparent soil component and randomly distribution of spores; its impossible to study the soil directly under the light microscope, so there are simples methods for this aim as:

I.2.1.1.1 Using of Contact Slide Method: for studying the natural distribution of in soil. For making this method, take clean glass slides and put them in different site in soil for
one week after that bring the slides, dry, fix them by flame then wash the slid and finally staining these slides for microscopic examination. This method using for studying the distribution of hyphae in soils but its not useful for identification the types of fungi due to not available of spores.

I.2.1.1.2 Soil Smear method: by using clean glass slides and press it in soil then stain these slides with lactophenol- Cotton Blue.

I.2.1.2 Indirect method

I.2.1.2.1 Dilution plate method: for quantitative studies but this method is not recommended for studying of fungi due to rapid and different types reproduction. Studying by this method is performed as:
- Set up 4 tubes containing 9 ml of sterile D.W.
- Weight 1 g of soil and put them into 9 ml of sterile D.W, mixing with using vortex. Dilution will be 1:10. Discard the tip.
- Repeat in this way with your dilutions down to $10^{-4}$.
- Using a fresh pipette tip and starting with the highest dilution, take 0.1 ml of suspension and transfer it to the surface of PDA.
- Spread out your dilution over the whole surface of the plate.
- Incubate the plate at room temperature.

I.2.1.2.2 Soil plate method: for isolate fungi that exist in hyphal shape around the soil. Studying by this method is performed as:
- Strewing 0.05 g of soil on nutrient media complementary with antibiotics.

I.3 Isolation from Infected Plant: The general procedure for isolation any type of fungi from plant materials are as follows:
- Wash plant materials under running tap water for at least 5- 10 min.
- For isolation from diseased plants, freshly infected parts may be selected and they are cut into tissue segments for less than 5 mm. From the aged, infected tissues, the more saprophytic fungi may be isolated.
- Sterilized plant tissues with solution hypochlorite or ethanol. Concentrations of chemicals may be different according to the samples used, but generally they may be soaked in 70% ethanol for 30 sec to 5 min.
- Uses sterile D.W to remove the effects of ethanol or sodium hypochlorite.
- Plant tissue segments placed on isolation media at appropriate temperature for 1-7 days.

I.4 Isolation of fungi from contaminated water: The procedure for this study as in the following:
- Bring contaminated water in sterile bottle with one remake don’t selling the bottle.
- Set up 4 tubes containing 9 ml of sterile D.W.
- Transfer 1 ml from the sample into 9 ml of sterile D.W, mixing with using vortex. Dilution will be 1:10. Discard the tip.
- Make this serial dilution like dilution plate method for soil.
• Finally Spread or streak out your dilution over the surface of the plate.
• Incubate the plate at room temperature.

![Image of fungal culture purification process.]

Fig. 3.1 Purification of fungal culture by streak plates

I.5 Sub culturing: make a subculture from old colonies of fungi as follows:
• Obtain a metal loop or needle and flame it until it turns red hot.
• Wait for about 10 seconds for the loop or needle to cool down.
• Using the loop or needle according to which you will transfer, remove an isolated colony of fungi.
• Aseptically transfer the cell colony into the nutrient media.
• Incubate the plate at room temperature for the next week.

II. Morphological characteristics and microscopic feature in the next week.
• Review all of the pervious roles and labs.

II. 1 Record your results in your lab sheet below and draw every fungal you will seen.

Fungus: -------------------------------------------- (genus/species)

Colony Morphology
• Obverse:-----------------------------------------------------------------------------------------------
• Reverse:------------------------------------------------------------------------------------------------

Microscopic view (describe AND draw hyphae, conidia, etc. Include pigmentation.)

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