

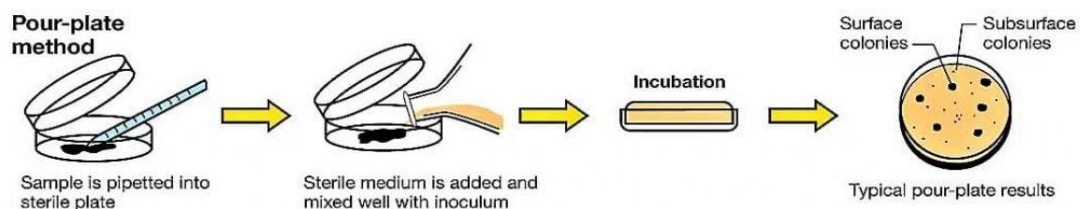
## Isolation of Fungi

### A. Isolation fungi from soil

#### a) Pour plate methods (Direct Isolation)

- 1- Weighed 0.1 gm of soil and placed it in Petri dish.
- 2- Poured the culture media in Petri dish near flame.
- 3- Moved the Petri dish with a circular movement right and left for mixing the soil with media.
- 4- Leave the plate until it solid, then incubated for 5 days at 25-28 °C.

Not: Can distribution the soil sample on the surface of solid culture media.



#### b) Indirect isolation:

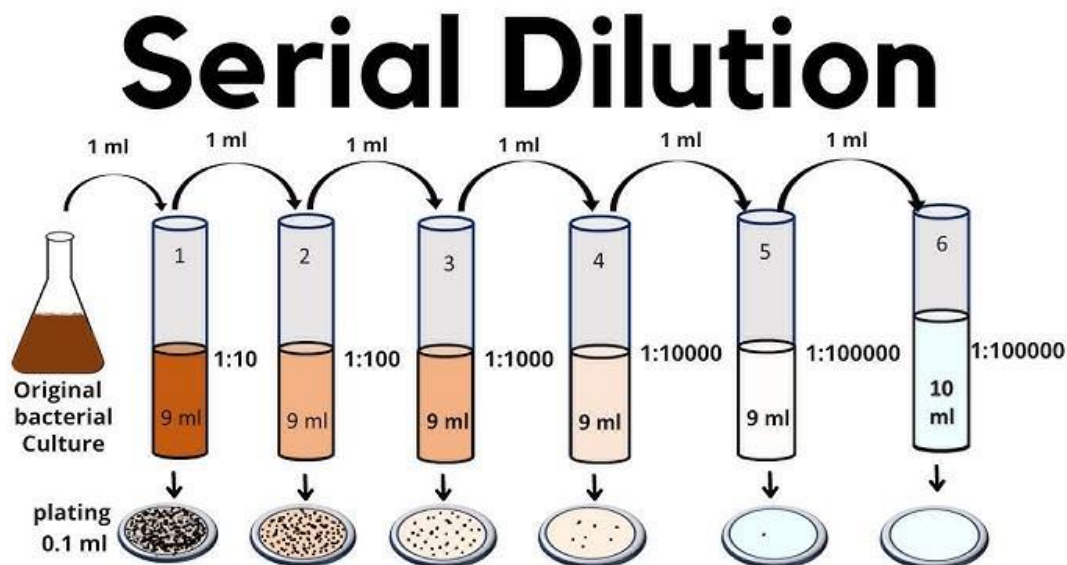
It's a modified method of poured plate methods, use to Purification fungi from bacteria.

- 1- Add 10 ml of culture media in Petri dish and left to solid.
- 2- Placed 0.1 gm of soil sample above the culture media.
- 3- Poured 5 ml of culture media on the plate and left to solid.

In this case hyphal fungi grow too high because its Aerobic organisms while the bacteria (anaerobic) stay within the culture media.

## c) Dilution method:

- 1- Weighed 1 gm of soil and placed it in test tube contain 10 ml Distilled water (DW) (stoke), Mixed it well.
- 2- Prepare 5 test tube each contain 9 ml of (DW).
- 3- Take 1ml of stoke to the first tube that will be first dilution 1/ 10 ( $10^{-1}$ ).
- 4- Take 1ml of the first dilution to the second tube that will be the second dilution 1/ 100 ( $10^{-2}$ ).
- 5- Take 1ml of second dilution to the third tube that will be the third dilution 1/ 1000 ( $10^{-3}$ ).
- 6- Take 1ml of third dilution to the fourth tube that will be the fourth dilution 1/ 10000 ( $10^{-4}$ ).
- 7- Take 1ml of fourth dilution to the fifth tube that will be the fifth dilution 1/ 100000 ( $10^{-5}$ ).
- 8- Placed 1 ml of each filtrate dilution in a Petri dish and then poured the culture media, moved the dish for mixing the sample with the culture media.
- 9- Leaf it until solid, then incubated for 5 days at 25-28°C. Not: The dilution (1ml) can be distributed on surface of solid culture media.



**B. Isolation fungi from air:**

- 1- Poured the media on Petri dishes and leave it until solid.
- 2- Open the dished in several different places for 30 min.
- 3- Incubated for 5 days at 25-28 °C.

**C. Isolation fungi from water:**

a) **Dilution method:** as mentioned earlier

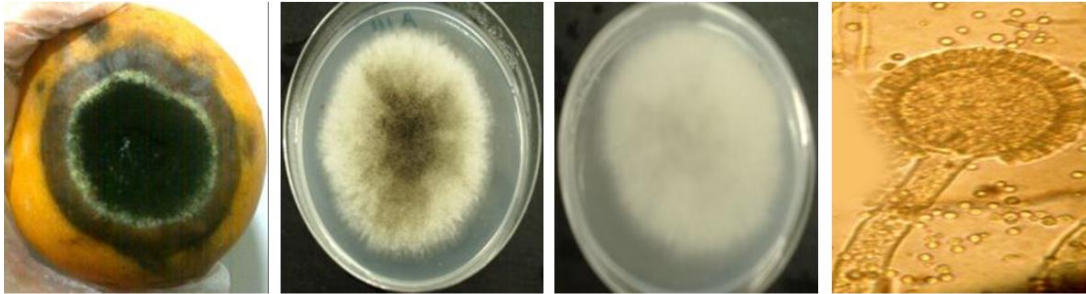
b) **Filtration methods:**

- 1- Filtrate the water sample using cellulose filter Placed in a sterile suppression.
- 2- After the passage the sample, Filter paper taken by sterile forceps and placed on surface of culture media then incubated the dishes for 5 days at 25-28 °C.

**D) Isolation fungi from fruits and vegetables:**

- 1- Placed the media into the plate and left it until solid.
- 2- Surface sterilization to rotten part by Sodium Hypochlorite or Potassium Permanganate (2%).

- 3- Washing the sample by (D.W) 2-3 times.
- 4- Transfer part rotten to the Petri dish by a sterile needle.
- 5- Incubated the dishes for 5 days at 25-28 °C.



(a) stages of Fungal infection in mango fruit (a), surface view of old colony on Potato dextrose agar (b),