LAB.3

ISOLATION AND NUMERATION OF SOIL MICROORGANISM

Soil is a suitable environment for a diverse of microbial community consists of bacteria, actinomycetes, molds, yeast, algae, and protozoa. Due to differences in nutritional requirements for different type of microorganism in the soil its essential to use different types of culture media for isolation and numeration.

1- **Nutrient agar**: for numeration of bacteria.

2- **Jensen's media**: for numeration of Actinomycetes. Actinomycetes isolate on Petri dishes are characterized by a dry or dusty or chalky colony. Also, it characterized by a distinctive earthy odor like the smell of rain when falls on dry soil.

3 - **Sabouraud agar**: for numeration of fungi.

Viable count

There are two main methods for bacterial counting: spread plate method and pour plate method

1- Spread plate: Spread plate technique is a method for isolation and numeration of microorganisms from soil. The technique makes it easier to quantify the bacteria in a sample.

Principle of Spread Plate Technique

The spread plate technique involves using a sterilized spreader with a smooth surface made of metal or glass to apply a small number of bacteria suspended in a solution over a plate. The plate needs to be dry and at room temperature so that the agar can absorb the bacteria

more readily. A successful spread plate will have a countable number of isolated bacterial colonies evenly distributed on the plate.

1- weigh out 1 g of soil sample and add to 99 mL of deionized water
2- Make a series dilution from a soil sample.
3- Pipette out 0.1 ml from the appropriate desired dilution series onto the center of the surface of an agar plate.
4- Dip the L-shaped glass spreader into alcohol.
5- Flame the glass spreader (hockey stick) over a Bunsen burner.
6- Spread the sample evenly over the surface of agar using the sterile glass spreader, carefully rotating the Petri dish underneath at the same time.
7- Incubate the plate at 37°C for 24 hours.
8- Calculate the CFU value of the sample. Once you count the colonies, multiply by the appropriate dilution factor to determine the number of CFU/mL in the original sample.

Determine No. of bacterial cells in soil sample according to the following equation:

No. of bacterial cells /1ml= No. of colonies *x* inverted dilution *x* 10

*Note: count plates which show only about 30-300 colonies

2- The pour plate method:

The pour plate method for counting bacteria from soil sample is more precise than the streak plate method, but, on the average, it will give a lower count as heat sensitive microorganisms may killed when they come contact with hot, molten agar medium.

Principle of pour plate Technique

In pour plate technique a successive dilution of the soil sample is added to the sterile Petri plates containing a melted (40-45 °C) agar medium, then thoroughly mixed by rotating the plates which are then allowed to solidify. After incubation, the plates are examined for the presence of individual colonies that growing throughout the medium.

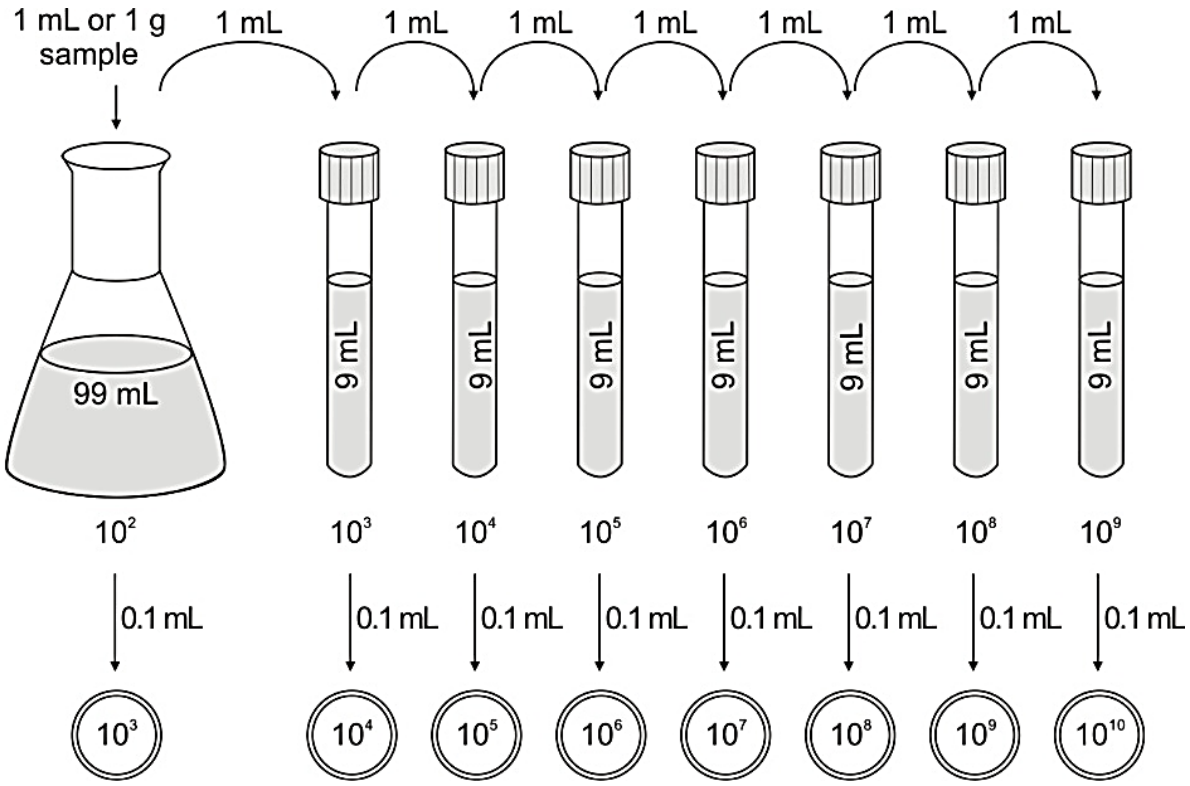
1- Put agar media in water bath in 45°c. to be liquefied.
2- Take a soil sample and make serial dilution as we describe above.

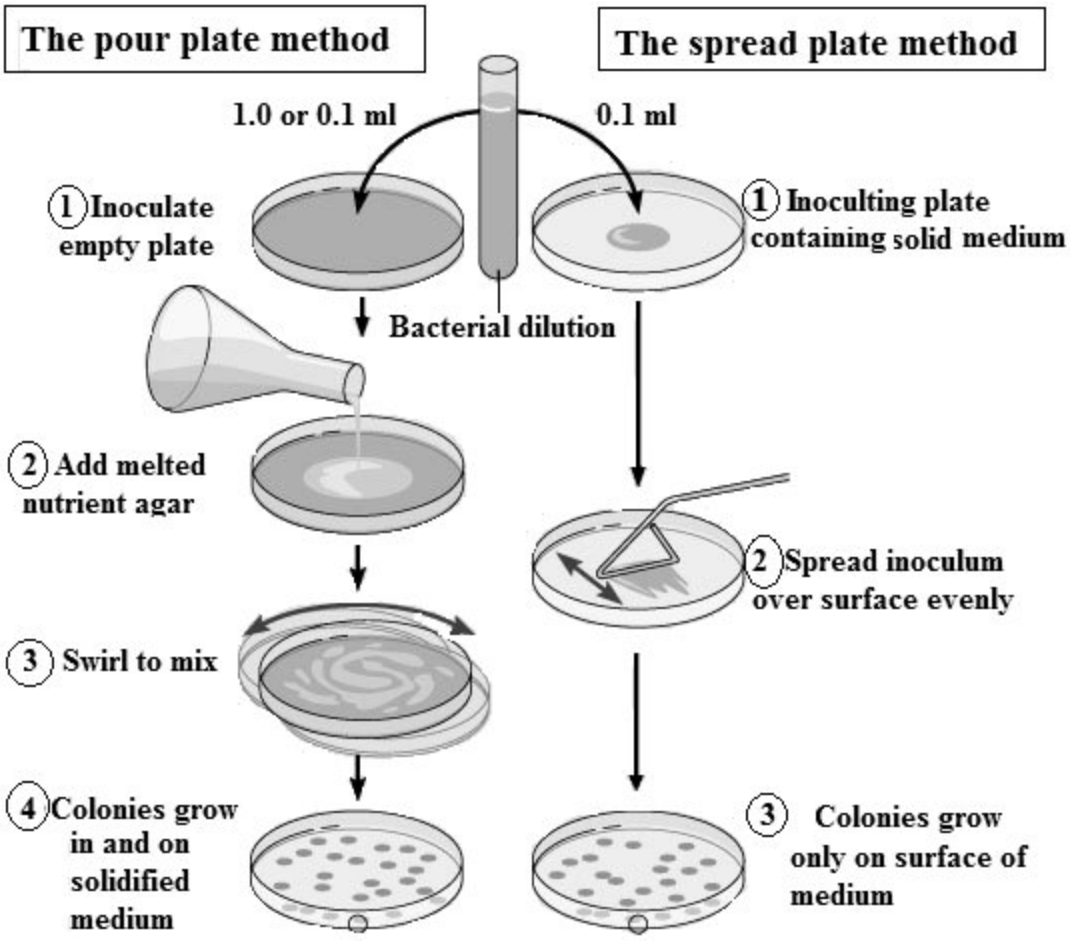
3- Transfer 1ml from last dilution by pipette to sterile petri dish.
4- Pour melted agar and mixed with the dilution sample.
5- Leave petri dish to solidify.
6- Incubate the plate at 37°c for 24 hours
   Determine No. of bacterial cells in soil sample from following equations:

*No. of bacterial cells /1gm moist soil = No. of colonies *x* inverted dilution

*No. of bacterial cells /1gm dry soil= $\frac{\text{No. of colonies x inverted dilution}}{\text{Dry weight of 1gm soil sample}}$

The unit of measurement here (CFU) Colony forming unit. where the colony may be the yields of the growth and multiplication of a single cell or more.





Rossi-Cholodny (Buried Slide) Technique

The distribution of microorganisms in soil is heterogeneous. Microbes need nutrients and water to survive and these resources are not evenly distributed in soil. The structure of soils is composed of particles of inorganic and organic matter and the pores in between these particles. The pore spaces may be filled with water or air. Bacteria are mostly found attached to particles growing in small micro-colonies wherever nutrients can be found. Filamentous organisms such as Actinomycetes and fungi make up much of the biomass. When they grow around soil particles they help to cement them into aggregates. These aggregates have their own internal pore spaces. The filaments also stretch between aggregates.

Often when soils are examined microbiologically, the native structure is destroyed and it is impossible to determine the original distribution of the microbes in their natural habitat. One way to visualize how microbes are distributed in soil is to use the soil contact slide method developed by Rossi (1928) and later Cholodny (1938) hence termed as Rossi-Cholodny Burried slide technique.
In this technique, glass slides are buried in soil and allowed to incubate. Bacteria and fungi attach to the glass as though it was a mineral particle and grow on the surface. When the slide is carefully uncovered and stained, the microbes remain in their original positions and their associations with particles and each other can be seen.

Procedures:

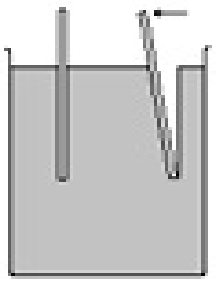
1- Weight 100 g of soil and placed in the clean container, if the soil is dry, moisten with water by adding 5 to 15 ml.

2- Make a slit in the soil and put gently 2- 4 clean microscope slides leaving at least 2 cm ($3/4_{th}$ is buried) protruding above the soil surface. The glass slide could be covered with semi solid media or not. Soil should be pressed gently around the slides to insure their contact.

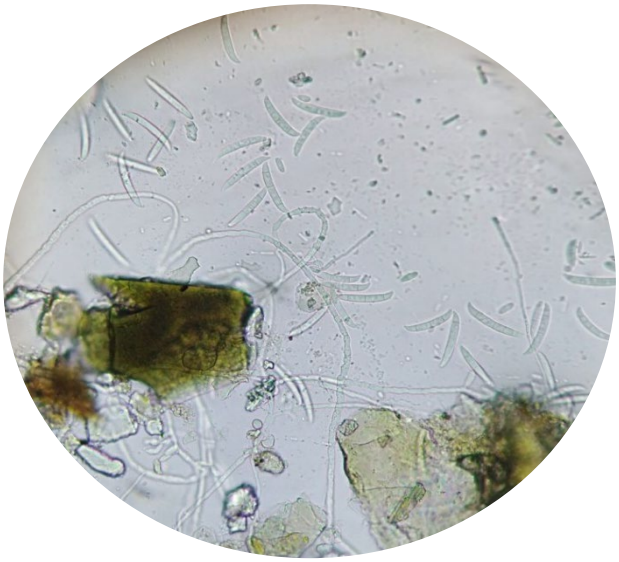
3 - Add enough water around the slide. Do not waterlog the soil.

4 - Cover the container and incubated at room temperature form one week to two weeks.

5 - Remove the slide by tilting it backwards so that the top of the slide is not scraped off as the figure bellow.



6 - Shake off any large soil particles and clean the bottom of the slide with a moist paper towel. Allow the slide to thoroughly dry.
7- Drying slide by flame for fixation
8 - Stain the slides by Gram stain or Carbol Fuchsin or Methyl Blue and washed with water and leave to dry.
9 - Wash off the excess stain and allow the slide to dry.
10 - View under the microscope. Look for fungal filaments, actinomycetes and bacterial micro-colonies.
11 - Record all observations.



* Macrospores of *Fusarium* spp. isolated from soil sample by buried slide method