Lab 3 Soil Microbiology

Isolation and Enumeration of Soil Microorganism

Soil is variable environment with divers‘ microbial community consists of bacteria, actinomycetes , molds ,yeast, algae and protozoa.

Necessary to use different types of culture media due to differences in dietary requirements for each type of microorganism to be isolate .

Note: culture media used the following, according to the type of microorganisms to be isolated:

1- Enumeration of bacteria used Nutrient agar .

2- Enumeration of Actinomycetes used Jensens media , characterized Actinomycetes isolated in dishes as dry and dusty or chalky. Also characterized dishes distinctive odor similar to odor earth after rain.

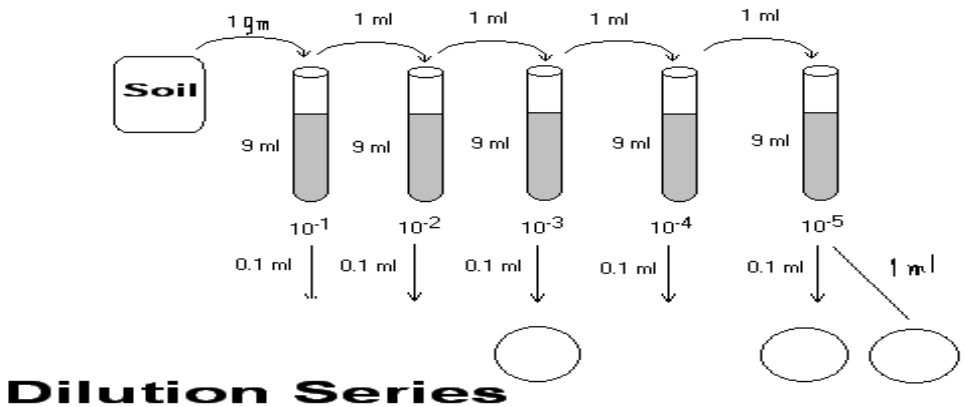
3 – Enumeration of fungi used sabouraud media.

There are two main methods of direct plate counting: spread plate method and pour plate method :

1-The spread plate method consists of evenly spreading the diluted sample over an agar plate. Using this method yields colonies that form on the surface of the agar.

procedure :

1. make serial dilution of microorganism sample in series of tubes containing D.W.
2. transfer 0.1ml from last dilution of microorganism culture by pipette.
3. put it on the centre of an agar plate.
4. moist spreader with alcohol and sterilize by flaming .
5. spread the sample on agar plat by spreader.
6. sterilize it again.
7. incubate the plate at 37°c for 24 hours, and then examine and count the present colonies distributed throughout the agar.



Dilution Series

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Note :

1- Count plates which show only about 30-300 colonies.

2- Used colony counter to enumerate the colonies .

Determine No. of bacterial cells in soil sample from equation:

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

2-The pour plate method, a volum of 1 ml of the diluted sample is put into a sterile petri plate, then melted agar is poured in and mixed with the sample. This method yields colonies that form colonies throughout the agar (growing both on the agar and in the agar , not just on the surface.

procedure :

1. Put agar media in water bath in 45°c. to liquefied .
2. Add 1gm of sample to first tube and make serial dilution from one to another tube.
3. transfer 1ml from last dilution of microorganism culture by pipette ,then Put in 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 hour

Determine No. of bacterial cells in soil sample from equation:

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

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

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.