Lec 9b ISOLATION ,ENRICHMENT,PRESERVATION

Microorganisms are present in all environments. The enumeration (counting) and isolation will be useful to study the diversity of microbes in that particular environment. The example environments are soil, water, rhizosphere, plants and animals internal and external parts, etc. To enumerate and isolate from different environments, there are many techniques are available. a. Direct microscope count method; b. Electronic cell count method; c. Microbial biomass estimation d. Measuring the microbial activity by some metabolites e. Spectrophotometer analysis are available to count the cells. The major disadvantage of the methods a,b are that the dead cell may also be counted ; c – very laborious and low precision; d and e – need very high standards.
An alternate easy and simple method to count and isolate the microorganisms from an environment is **serial dilution – plating technique**.

The technique is based on the principle that complete detachment and dispersion of cells from the environment will give rise to discrete colonies when incubated on a Petri plate containing media. The assumptions that underlie this technique are

- Complete dispersion of sample
- Suitable growth media for the organisms
- No interaction between organisms on the media

- **Bacteria** are prokaryotic unicellular organisms. The bacterial population of soils is dominated by species of *Pseudomonas*, *Arthrobacter*, *Bacillus* and others. Dilution plate techniques are used to study the relative abundance of soil bacterial types and the changes in population density
- **Fungi** are eukaryotic organisms with a filamentous growth form. Our knowledge of soil fungi is derived primarily from dilution and plating techniques. These methods are in favour of rapidly growing and sporulating organisms, and consequently most of the fungi identified by these techniques are Fungi Imperfecti (*Penicillium* and *Aspergillus sp.*).
- **Actinobacteria** are considered true bacteria but they have a filamentous growth habit composed of hyphae approximately 1.0 µm in diameter. The species most likely to be observed are members of the genus *Streptomyces*.

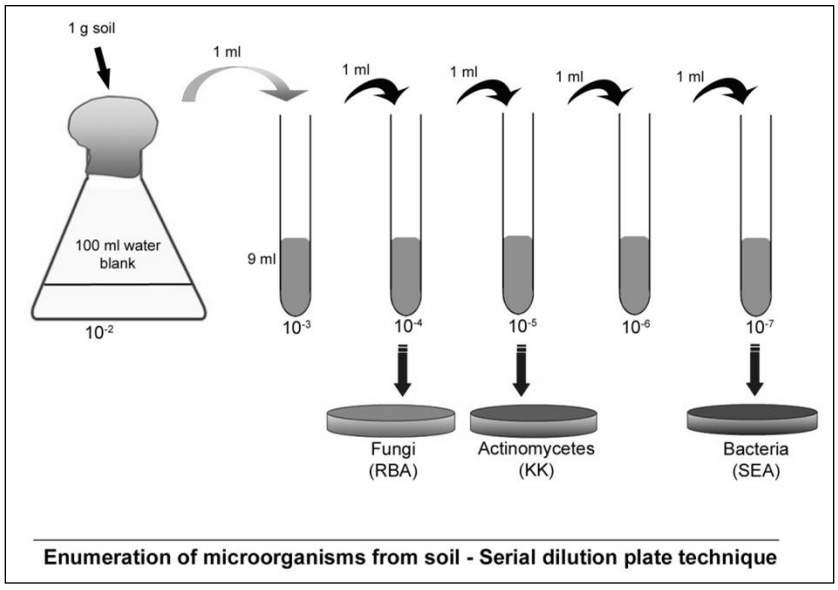
Procedure:

Step 1: Serial dilution & Plating

- Weigh one g of soil sample and put it in 100 ml sterile water blank
- Shake well for 15 min for complete dispersion (*This gives 10^{-2} dilution)*
- Transfer one ml of the suspension to 9 ml water blank *(This gives 10^{-3} dilution)* likewise serially dilute upto 10^{-7} dilution
- Based on the sample and previous experiences, appropriate dilutions were used for plating appropriate organisms

(*Note: Normally 10^{-7} or 10^{-8} can be used for bacteria; 10^{-3} or 10^{-4} for fungi and 10^{-4} or 10^{-5} for actinomycetes can be used if soil is used for enumeration)*

- Transfer 1 ml of appropriate dilutions to Petri dishes
- Maintain 2 or 3 replications for each dilution
- Pour the melted and cooled media (just before solidification) of about 15 ml to each petridishes with the suspension and mix well by rotating clock wise and anti clock wise for 3 or 4 times and keep it as such for complete solidification
- Label the organism, media name, date, dilution, batch no., on the bottom of the plate (write on the edge of the plate for easy count)
- Incubate the plates in inverted position at room temperature for 2 -7 days
- Observe the bacterial colonies after 2 days; fungi – 3 to 5 days; actinomycetes – 7 days (*Note: while counting actinomycetes, avoid counting the bacterial colonies, by experience)*
- Observe the shape, consistency, colour like some colony characters of each group of microorganisms
- Count the colonies per plate and express the population in terms of **colony forming units (cfu)**



Enumeration of microorganisms from soil - Serial dilution plate technique

Step 2: Determination of moisture content

- Record the weight of a container (beaker or Petri dish) and label
- Weigh 10 g of soil sample and record the total weight of the sample + container
- Dry the samples in oven at 105°C for 48 h
- Record the weight of the container + sample
- Record the dry weight of the soil sample (by deducting the total weight minus container weight)
- Calculate the moisture per cent and dry weight of the soil by following the formula

$$\text{Moisture per cent (\%)} = \frac{\text{wet weight (g)} - \text{dry weight (g)}}{\text{dry weight (g)}} \times 100$$

dry weight (g)

Step 3: Calculation

$$\text{Dry weight (g) of the soil taken for enumeration} = \frac{\text{wet weight taken for enumeration (g)}}{(\text{moisture\% } / 100) + 1}$$

Calculate the population of each group of organism using following formulae:

$$\text{Population (CFU per g dry weight of the soil sample)} = \frac{\text{mean cfu x dilution factor}}{\text{dry weight of soil taken}}$$

Where, Dilution factor = 1/ dilution

Disadvantages

There are many potential inaccuracies when using the dilution plate technique which result in an underestimate of the total viable population of cells. The factors responsible for the underestimation are:

- clumps of cells remain aggregated or attached to soil
- cells are killed in the dilution medium
- spores fail to germinate
- adsorption of cells on pipette walls and
- high selectivity of the plating medium and incubation conditions

It is generally accepted that plate counts account for < 10% of the total population.