Isolation of Microorganisms from Soil

Dr. A. Saha.
Assistant Prof. in Botany(St-III)
Surendranath College, Kolkata

Introduction

- The physical and chemical properties of the soil and soil fertility determine the nature of the soil environment in which these microbes occur.
- The largest number of microbes occurs in the top layer of the soil at a depth of 5-10 cm.
- In deeper layers(1.5-5m),the no. of microbes is reduced.
- In cultivable lands, microbes occur in large numbers.

- Besides composition of soil, climatic factors such as available moisture, degree of aeration, temperature and pH and agricultural practices also affect the distribution of microorganisms in the soil.
- Microbes occurring in soil are called soil microflora which are as follows

1. Bacteria

- They are the most frequent group of microorganisms present in the soil and constitute half of the total biomass.
- 1 gm fertile soil contains as many as 109 bacteria.
- Some common bacterial are the species of Pseudomonas
- Arthrobacter
- Achromobacter

- Bacillus
- Clostridium
- Micrococcus
- Flavobacterium
- Chromobacter
- Chromobacterium
- Mycobacterium etc.

- Escherichia occurs in soil due to sewage contamination.
- In cellulose rich environment celluloytic bacteria like Cytophaga, Sporocytophaga are found.
- Chemosynthetic autotrophic bacteria like Thiobacillus,Ferrobacillus,Nitrosomonas,Nitobacter.

2.Actinomycetes

- They are abundant in the soil rich in decomposed organic materials.
- They are responsible for the earthy smell of a freshly ploughed soil.
- Streptomyces
- Micromonospora
- Nocardia

3.Microfungi

- The quality and quantity of organic materials present in the soil and depth of the soil influence the fungal population
- Aspergillus
- Botrytis
- Penicillium
- Alternaria
- Fusarium

- Rhizopus
- Pythium
- Alternaria, Aspergillus,Cladosporium and Dematium are helpful in the preservation of organic materials.

4.Microalgae

- Occur on the surface of moist soils where sufficient light is available.
- Helpful for soil conservation and improving soil structure
- Chlorella
- Aphanocapsa
- Anabaena
- Nostoc
- Scytonema, etc.

5.Prortozoa

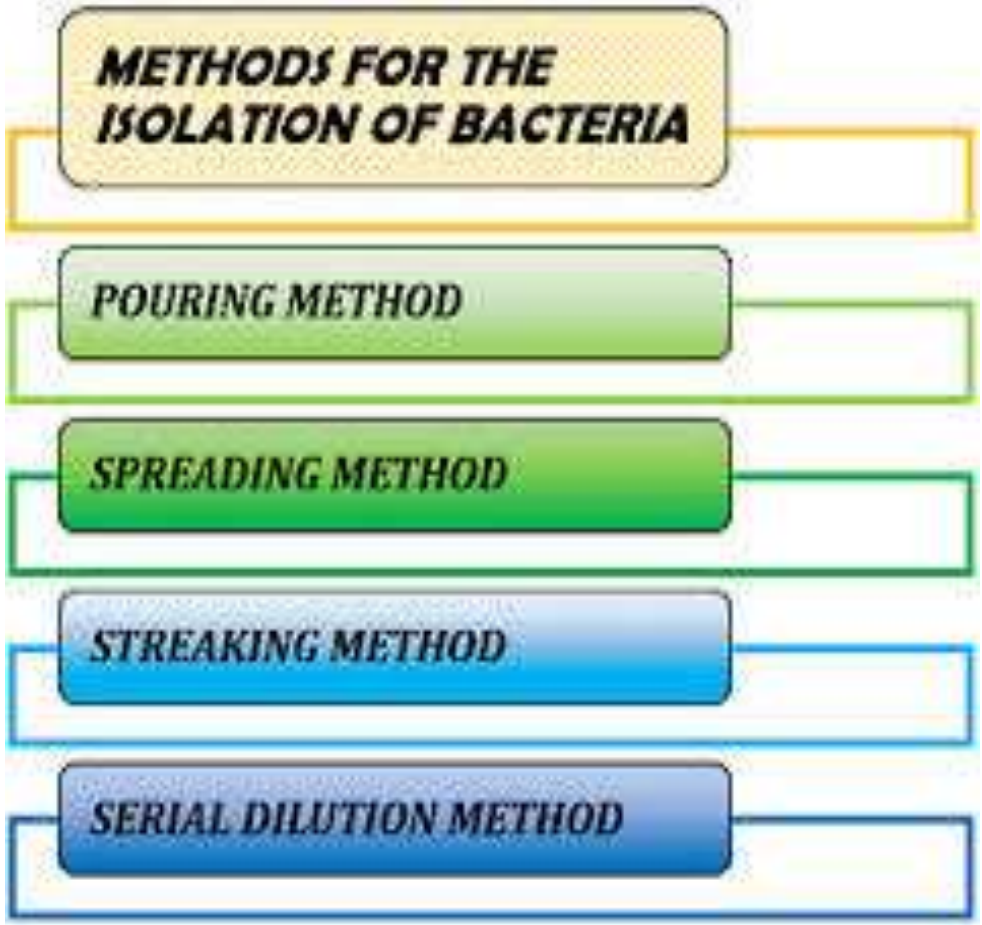
- Occur in great no. in the upper layer of the soil and have direct effect on the bacterial population as they ingest bacteria.
- Maintains equilibrium of the microbial flora in the soil.
- Allantion
- Bodo
- Monas
- Spiromonas
- Amoeba
- Vorticella, etc.

6.Viruses

- They are present in the least number in the soil.
- Bacteriophages ingest bacteria, actinomycets and some viruses infect the fungi present in the soil.

General Microbiology Laboratory

The Serial Dilution Method of Bacteria Enumeration


METHODS FOR THE ISOLATION OF BACTERIA
POURING METHOD
SPREADING METHOD
STREAKING METHOD
SERIAL DILUTION METHOD

Serial Dilution is one of the old method which is in use for the isolation of bacterial colony.

SERIAL DILUTION METHOD-

- This method is commonly used to obtain pure cultures of those microorganisms that have not yet been successfully cultivated on solid media and grow only in liquid media. A microorganism that predominates in a mixed culture can be isolated in pure form by a series of dilutions.

- The inoculum is subjected to serial dilution in a sterile liquid medium, and a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution.

Isolation

Isolation of bacteria from soil sample

Materials:

1) Samples (Soil, Tap water, Food, Slag, Milk) .. etc
2) Saline solution or Distilled water
3) Nutrient Agar
4) Beaker
5) Flask
6) Graduated cylinder
7) Test tubes
8) Test tubes stand
9) Media plates
10) Electric balance
11) Aluminum foil
12) Cotton
13) Micro peptide
14) Bumer
15) Wire loop
16) Ethanol
17) Para film
18) Autoclave
19) Incubator
20) LFH
21) Marker

PROCEDURE:

1) Collected desire Samples (Soil, Tap water, Food, Slag, Mild) from the environment
2) Take 8 test tubes and take 10 ml of saline in 1st test tube and take 9ml of distilled water in each the remaining test tubes and then labeled each test tubes.

3) Take 1 gram of Samples **(Soil, Food, Slag, Milk)** and make a solution in the 1st test tubes which having 10 ml of Saline solution. Which will called Master test tube.
4) Distribute the sample solution from the **1st** test tube(master test tube) in the remaining test tubes.

Take 1 mal of sample solution from **1st** test tube (Master test tube) and put in the **2nd** test tube.

Take 1mal of sample solution from **2nd** test tube and put in the **3rd** test tube.

Take 1 mal of sample solution from **3rd** test tube and put in the **4th** test tube.

Take 1 mal of sample solution from **4th** test tube and put in the **5th** test tube.

Take 1 mal of sample solution from **5th** test tube and put in the **6th** test tube.

Take 1 mal of sample solution from **6th** test tube and put in the **7th** test tube.

Take 1 mal of sample solution from **7th** test tube and put in the **8th** test tube.

5) Prepared Nutrient Agar media (NA) .
6) For the purring the samples in the media plates we use **Purring Plate Method .**
7) By using micro peptide We take **.5 ml** of sample solution from the desire test tubes and purring on the media plates by **Purring Plate Method**.
8) First we purring the sample solution in the test tubes and then we purring the Nutrient Agar in the plates. And labeled the plats also.
9) Allow the plates to solidified and then we replaced the inoculated media plates in the incubator at **37 C** for **24** hours.
10) After 24 hours we absorb the cultured plates, the growth of microbes will be appeared on the media plates.
11) Next we perform sub culturing for the isolation of pure culture.
12) Again we prepare new fresh Nutrient Agar, purring the media in the plates. Allow the plates to solidified and then we pick up a single colonies from each growth cultured plates and inoculated on fresh Nutrient Agar plates by using striking method.

13) After striking replaced the inoculated plates in the incubator at **37** C for **24** hours.

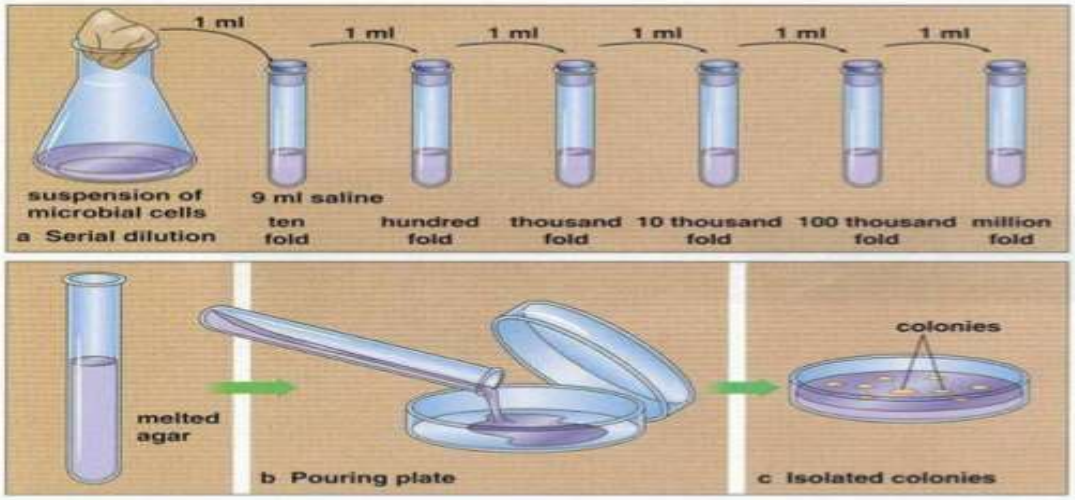
14) After 24 hours a pure, clear and visible colonies will appeared on each plates.

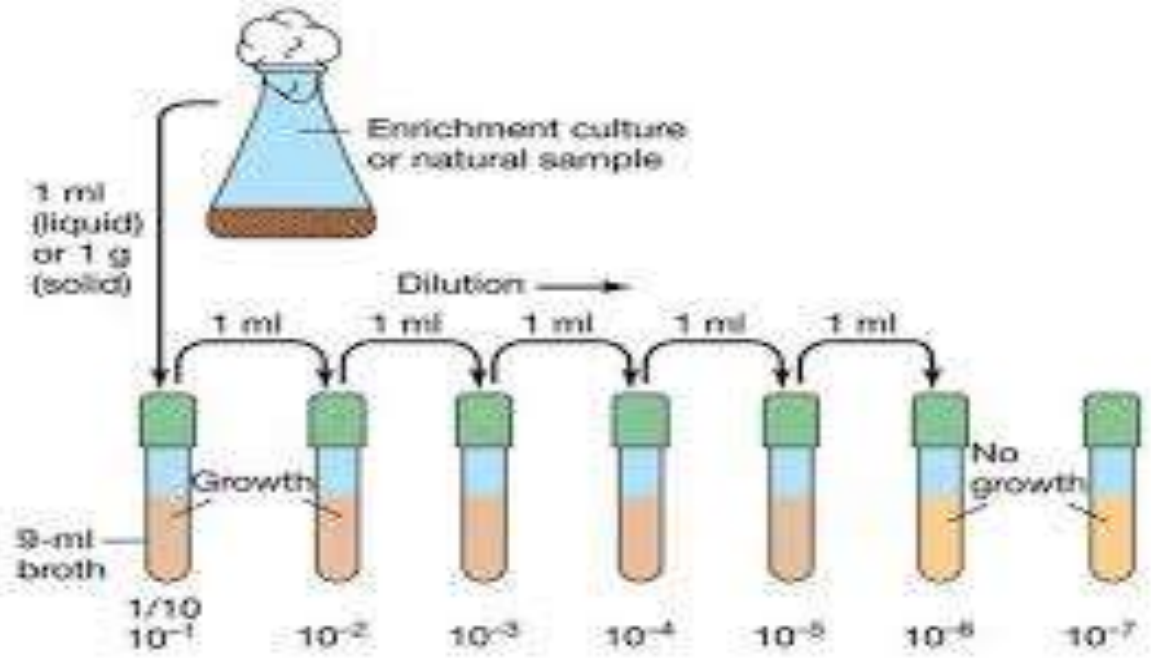
15) **Next** we use these pure culture for gram staining as well as for biochemical tests for the conformation and identification of the isolated bacterial colonies.

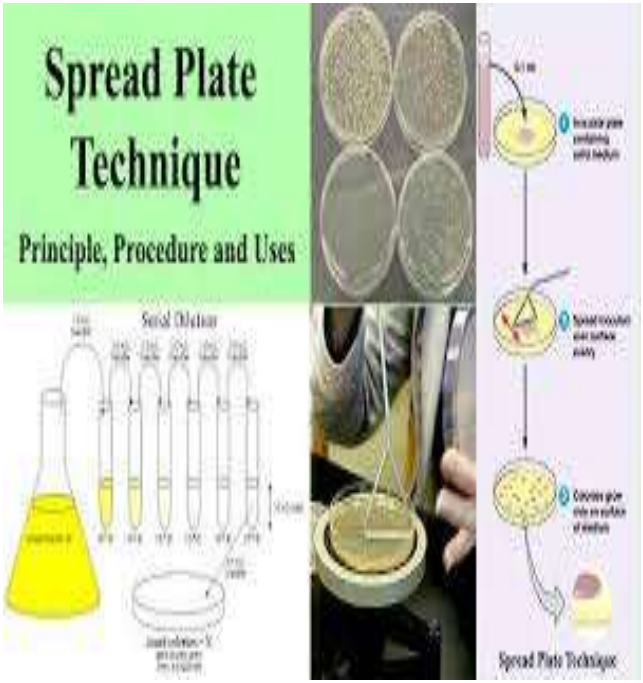
Note:

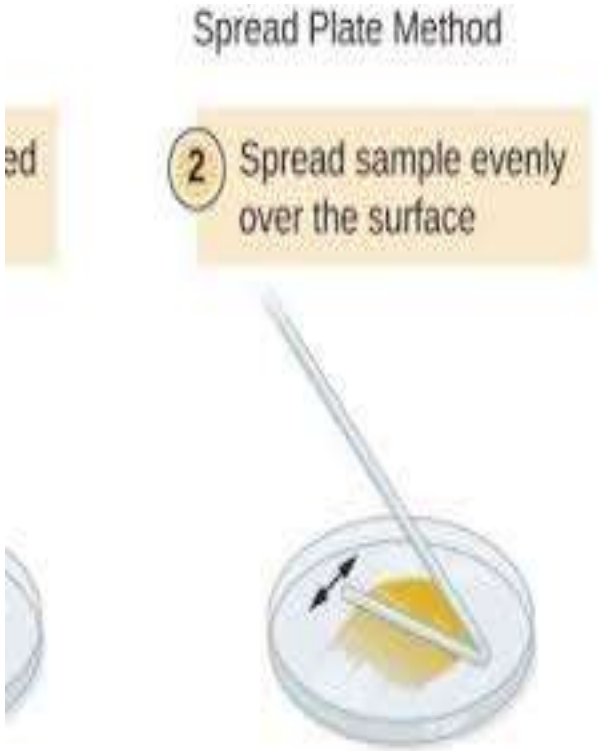
As the same method we can isolated microbes from slag , food and milk samples.

We use the same method and the same procedure for the isolation of microbes from these samples.

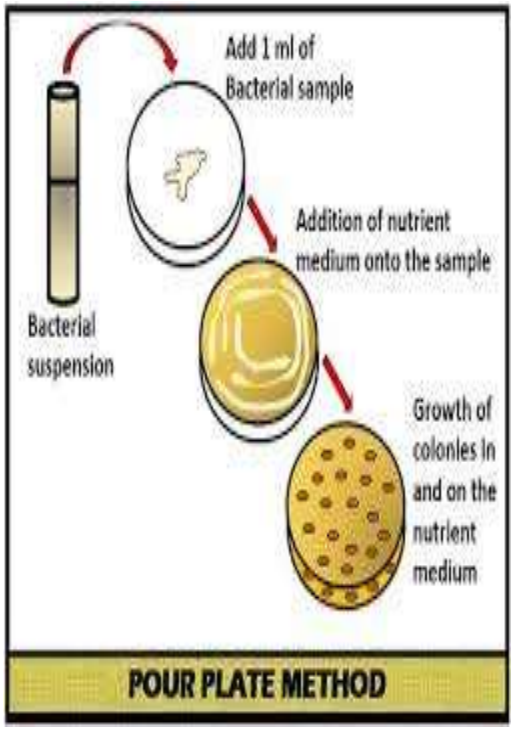


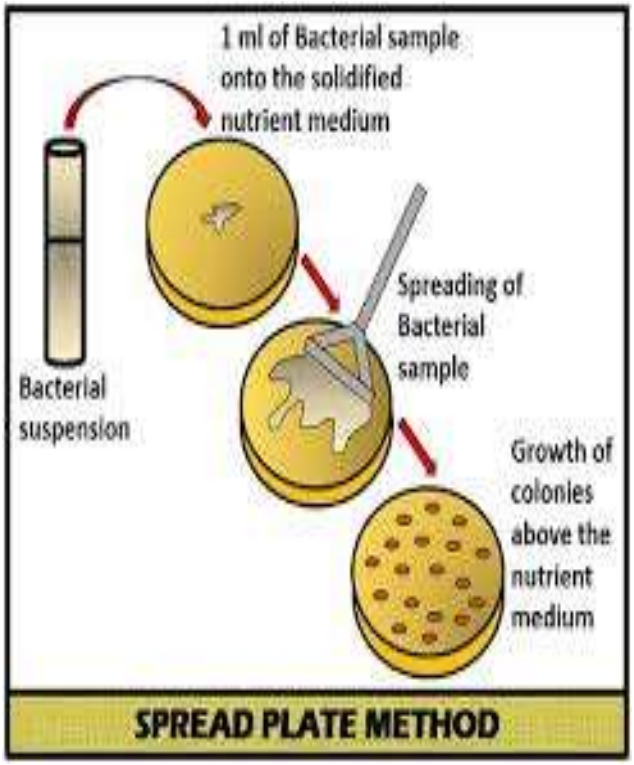

Enrichment culture
or natural sample
1 ml
(liquid)
or 1 g
(solid)
Dilution
1 ml
1 ml
1 ml
1 ml
1 ml
Growth
No
growth
9-ml
broth
1/10
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
10^{-6}
10^{-7}

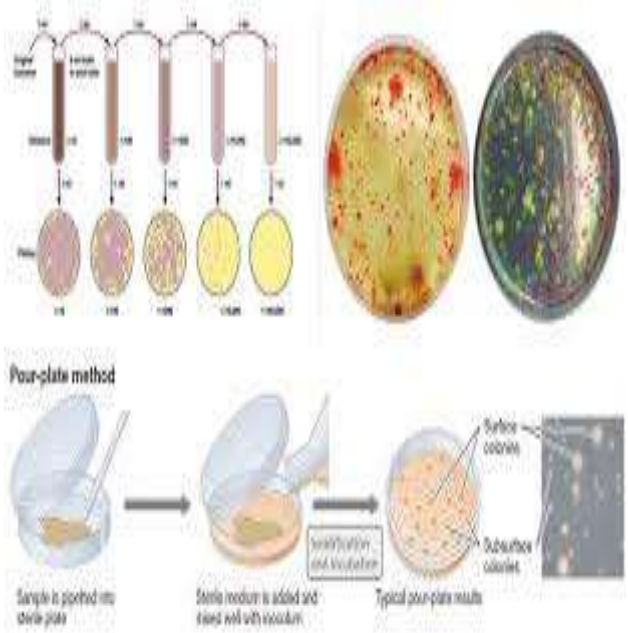

Spread Plate
Technique
Principle, Procedure and Uses
Spread Plate Technique

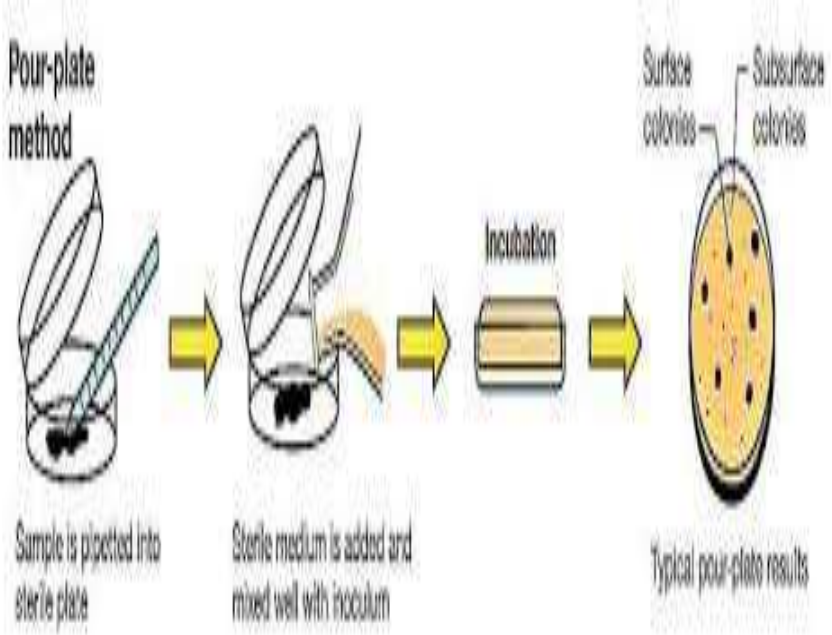

Spread Plate Method
2
Spread sample evenly
over the surface

Comparison

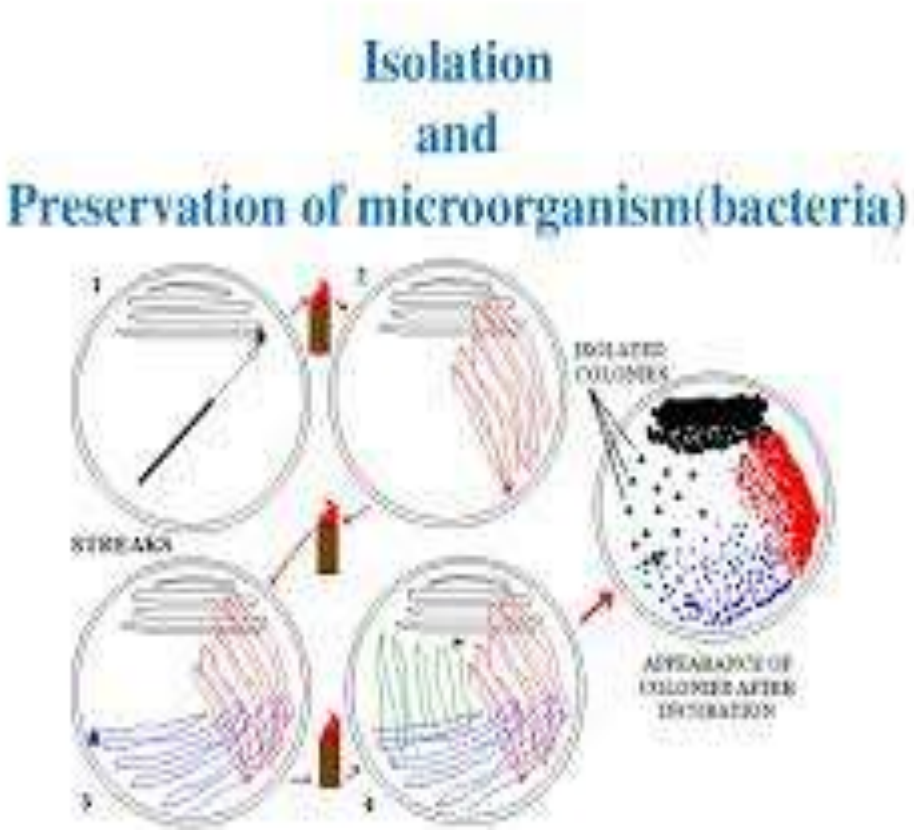

Add 1 ml of
Bacterial sample
Addition of nutrient
medium onto the sample
Bacterial
suspension
Growth of
colonies in
and on the
nutrient
medium
POUR PLATE METHOD

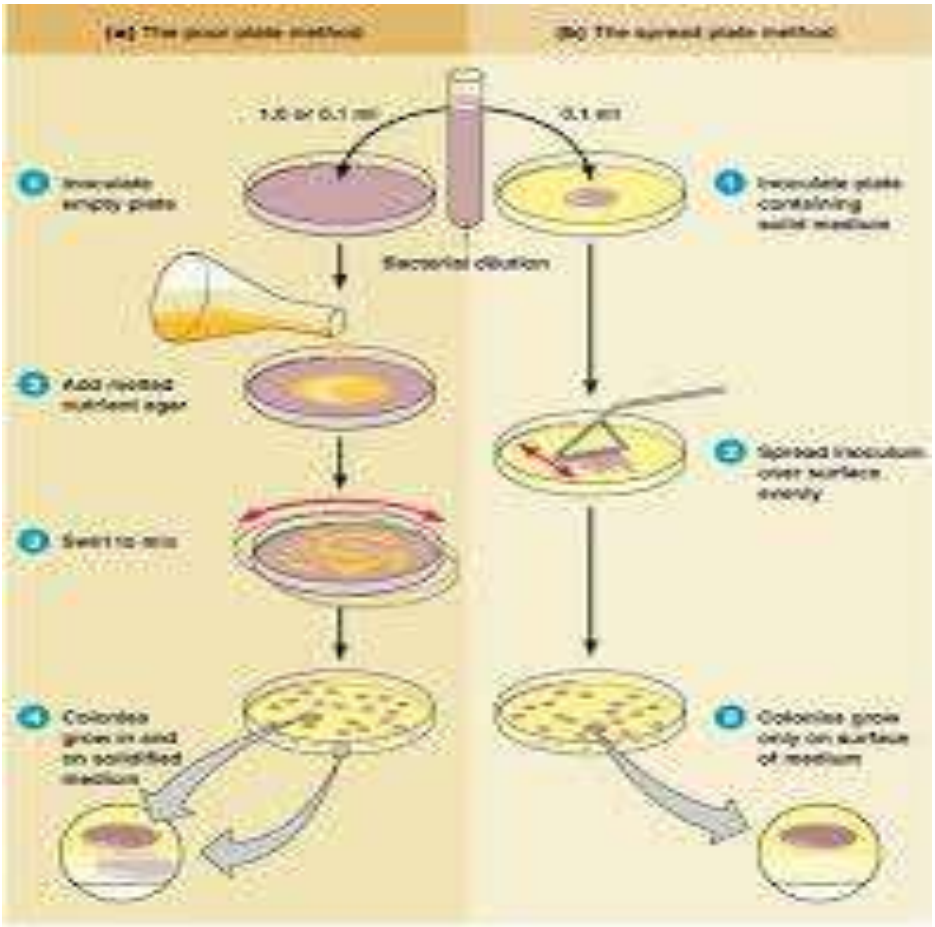

1 ml of Bacterial sample
onto the solidified
nutrient medium
Spreading of
Bacterial
sample
Bacterial
suspension
Growth of
colonies
above the
nutrient
medium
SPREAD PLATE METHOD

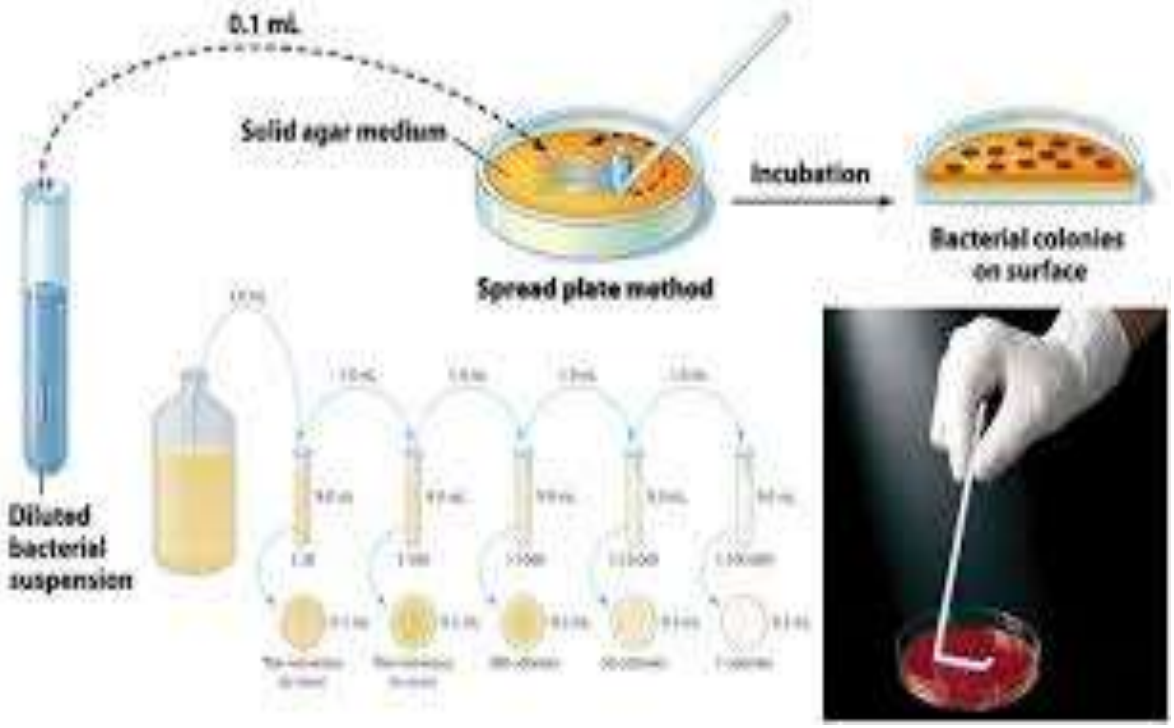

Pour-plate method
Surface colonies
Subsurface colonies
Sample is pipetted into sterile plate
Sterile medium is added and mixed well with inoculum
Typical pour-plate results


Pour-plate method
Surface colonies
Subsurface colonies
Incubation
Sample is pipetted into sterile plate
Sterile medium is added and mixed well with inoculum
Typical pour-plate results

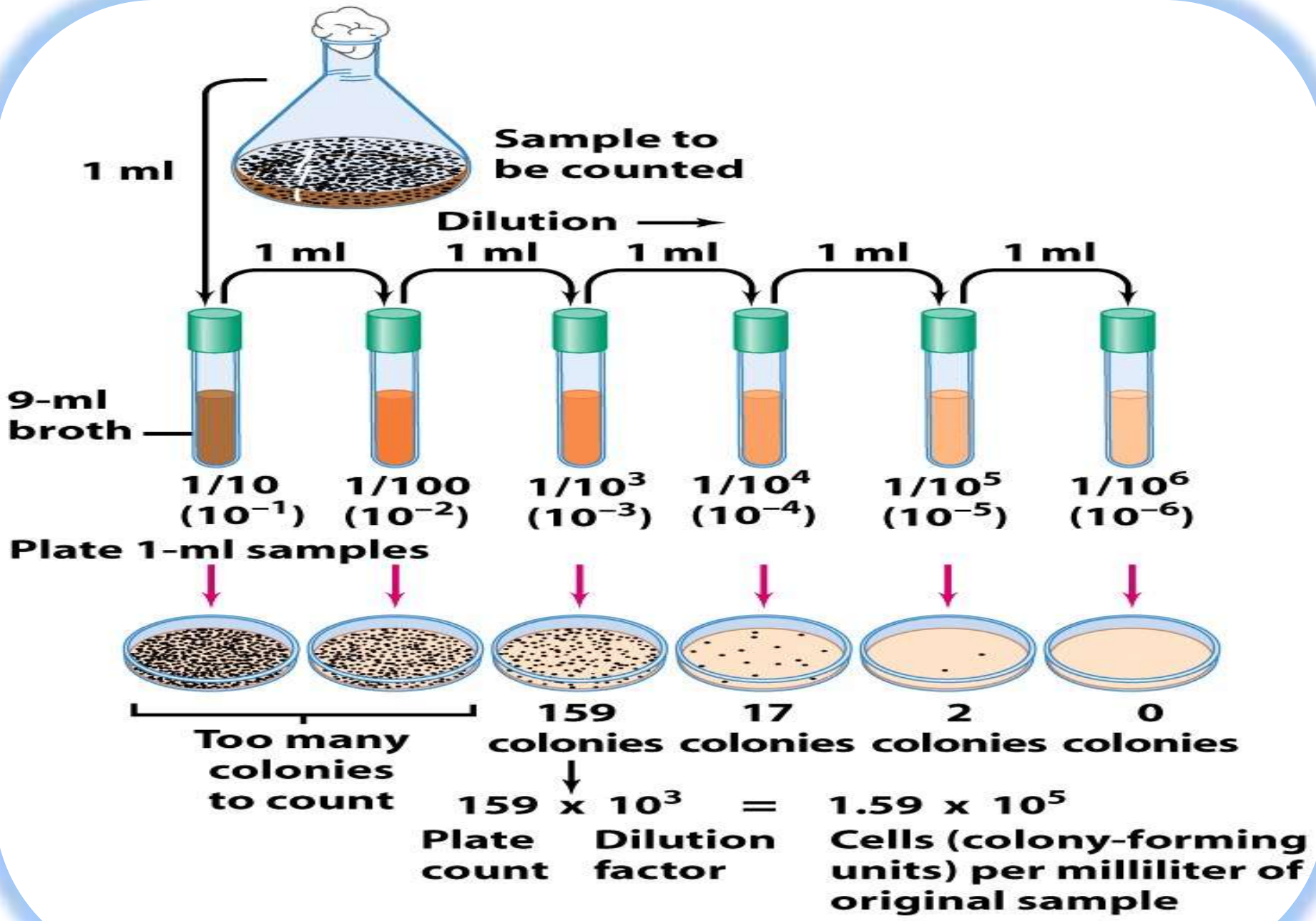
Streaking Method






0.1 mL
Solid agar medium
Incubation
Bacterial colonies on surface
Spread plate method
Diluted bacterial suspension

A plate showing microorganisms isolated from soil sample



ure 6-11 Brock Biology of Microorganisms 11/e
06 Pearson Prentice Hall, Inc.

References

.Internet Archives

. Pelczar, M. J. Jr. Chan. E. C. S. and Kriog, N. R. – Microbiology. Mc Graw Hill Book Company.

.Atlas, R. M. (recent Edition) – Principle of Microbiology, W. E. B. Mc Graw Hill

. Prescott, L. M. Harley, J. P. and Klein, d. A. – Microbiology. W. C. Brown Publishers, England.

. Schelegel, H. G. – General Microbiology. Cambridge University Press.

. Madigan, M. T. Martins, J. M. and Parker, J. – Biology of Microorganisms. Prentice Hall International Inc.