

# ISOLATION OF BACTERIA

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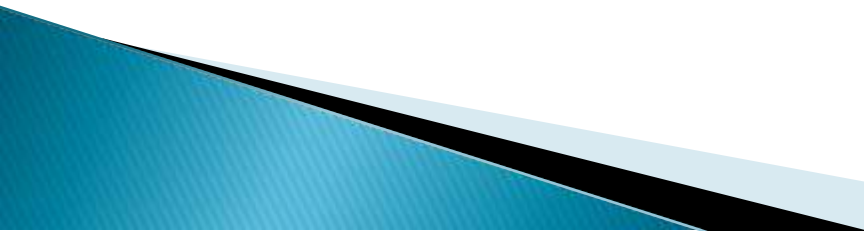
Ranjan Parida

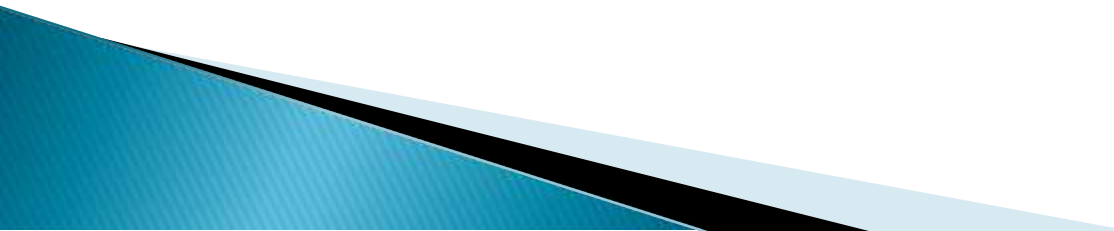
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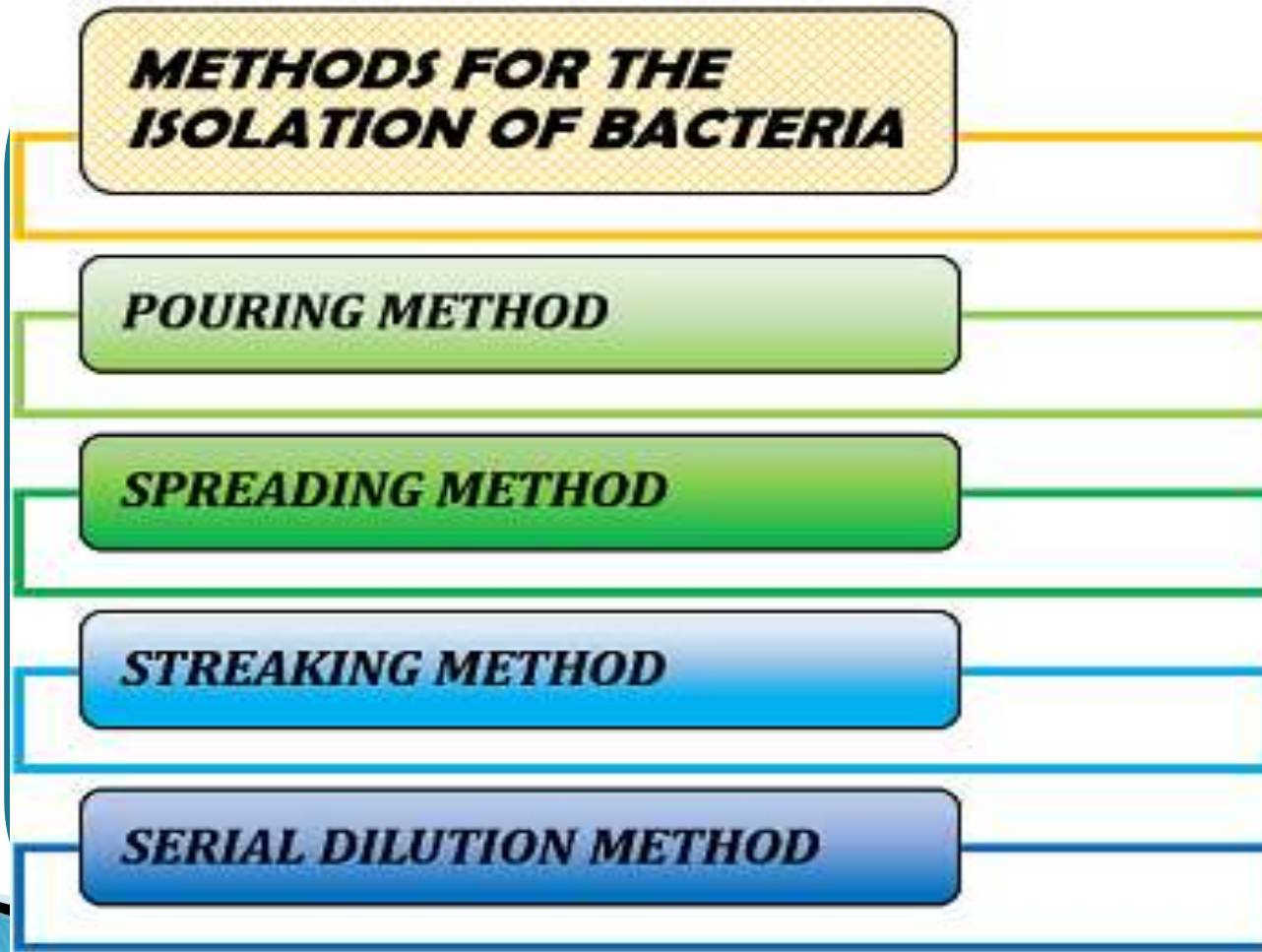
Isolation of bacteria is a primary method to separate different groups of microorganisms. It is the method which helps us to differentiate different groups of organisms. It is the method, which allows us to discriminate different groups of bacteria on the basis of **“Growth Pattern”**.

Different bacteria grow differently on the different nutrient medium based on their growth requirements and the other factors like temperature, pH, oxygen availability etc. Isolation of bacteria is an important step for the identification and differentiation of the bacteria.



- ▶ Definition
  - ▶ Isolation of bacteria can be defined as the technique of separating **one species** of bacteria from the **mixed culture** of bacteria by different plating methods like pouring, spreading, streaking and serial dilution.
  - ▶ In simple words, isolation of bacteria can be defined as the method of obtaining a “**Pure culture**” which is devoid of any contamination and other related microorganisms.
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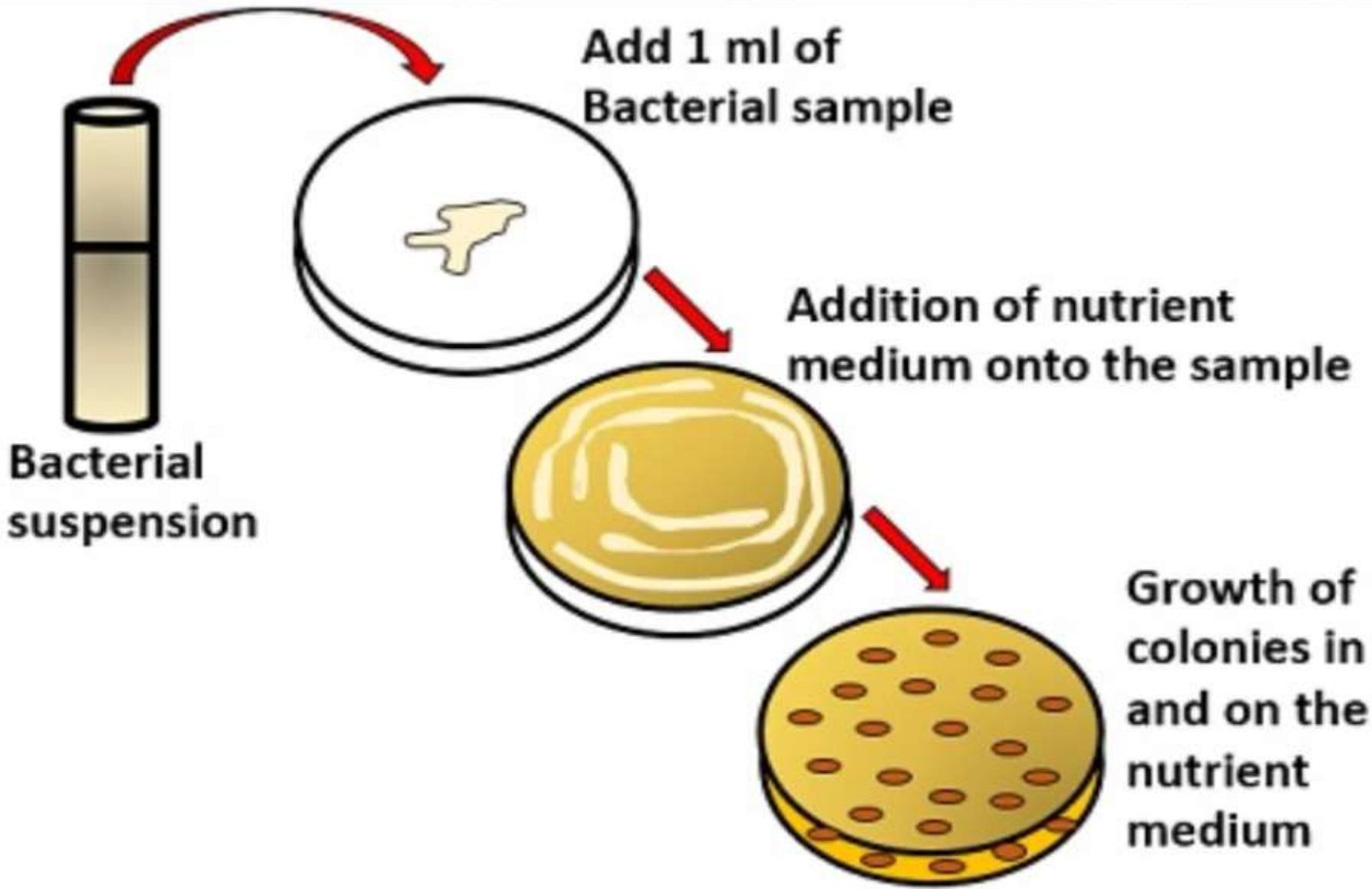
- ▶ Isolation Methods
- ▶ There are several methods for the isolation of bacteria, which includes:



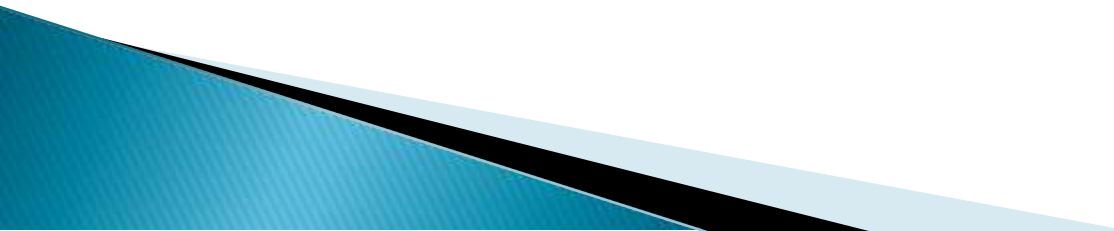
## ▶ Pouring Method

Pouring is a simple method for the separation of bacteria. In the pouring method, the bacterial suspension which carries the huge bacterial population is taken.

By the help of pipette, take 1ml of a bacterial sample into the sterile Petri plate. For the growth of bacteria, there should be some nutrient source like carbon and nitrogen. Therefore, the most common Agar nutrient medium is firstly prepared and then added to the Petri plates containing the bacterial sample.



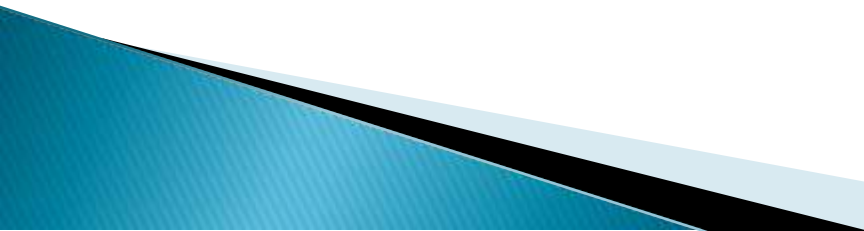
## **POUR PLATE METHOD**

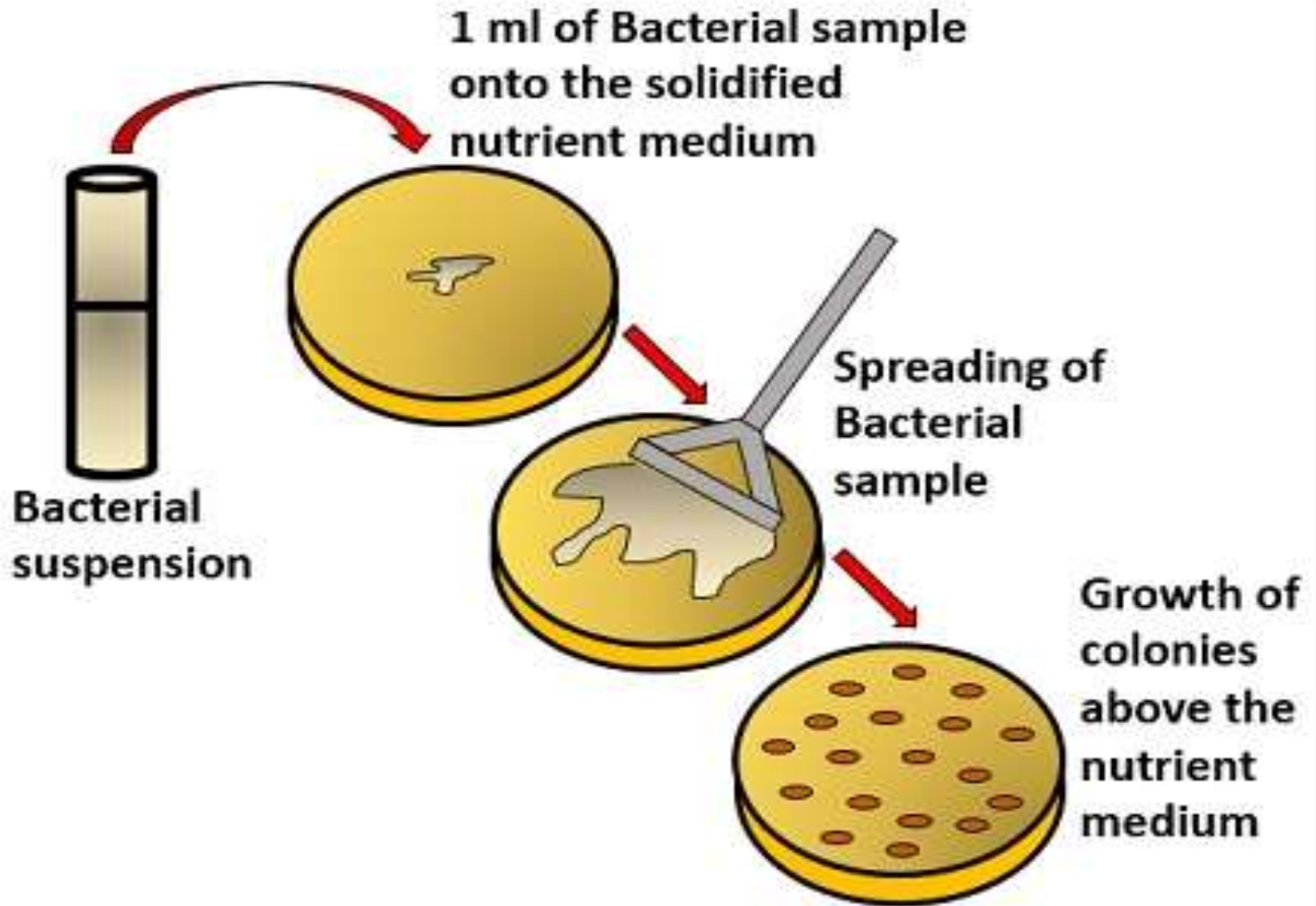
- ▶ For the uniform distribution of the sample and the media, **rotate the plates in the clockwise and anticlockwise direction.** Before keeping the Petri plates in the incubator, allow the culture plates to solidify.
  - ▶ For the proper growth of bacteria, keep the culture plates in the **incubator at the temperature of 35-37 degrees Celsius for the maximum period of 48 hours.** After the **incubation period of 24-48 hours,** we can see the growth of bacterial colonies.
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# Disadvantages of Pour plate method

1. The microorganisms are trapped beneath the surface of medium when it solidifies. Hence, surface as well as subsurface colonies are developed and it is very difficult to isolate and count the subsurface colonies.
2. This method is tedious, time consuming and requires skill.
3. The microorganisms are subjected to hot shock because liquid medium is maintained at 45°C temperature.
4. This method is unsuitable for isolation of psychrophile bacteria.

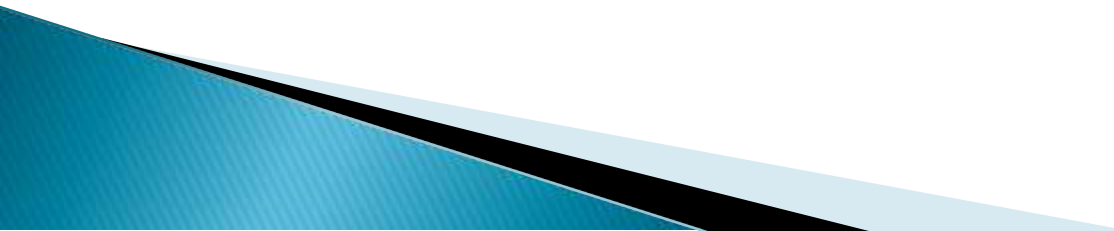


- ▶ Spreading Method
  - ▶ Spreading method is again a very simple method to perform isolation of bacteria. It differs from the pour plate method, add the nutrient medium first before the addition of the bacterial sample.
  - ▶ The nutrient medium is added to the sterile Petri plates and then allowed to solidify. After the solidification of the nutrient media, add 1ml of the bacterial suspension over the surface of solid media.
  - ▶ For the uniform distribution of bacteria over the surface of solid media, take the spreader of T or L-shape to spread the bacterial suspension evenly. After that, incubate the culture plates for 35-37degrees Celsius for 24 to 48 hours.
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## **SPREAD PLATE METHOD**

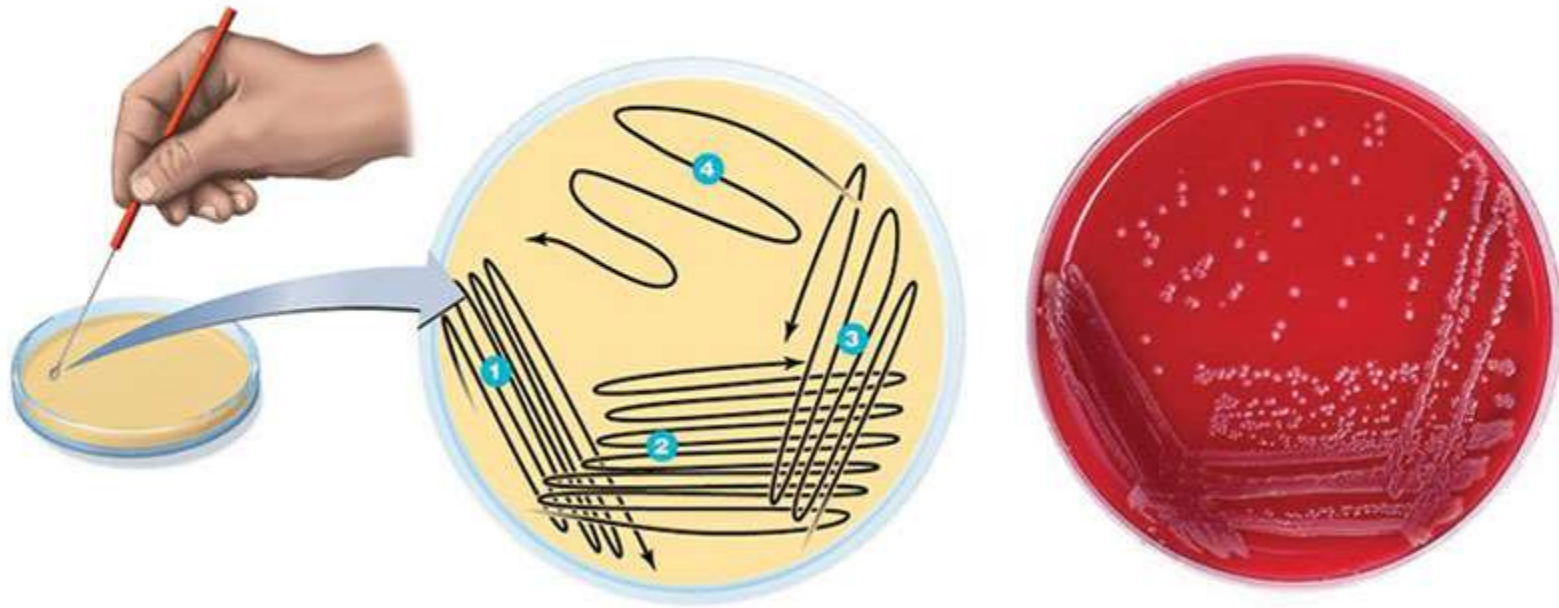
## Advantages of spread plate method

- ▶ It is a simple method.
  - ▶ In this method only surface colonies are formed.
  - ▶ Micro-organisms are not exposed to higher temperature.
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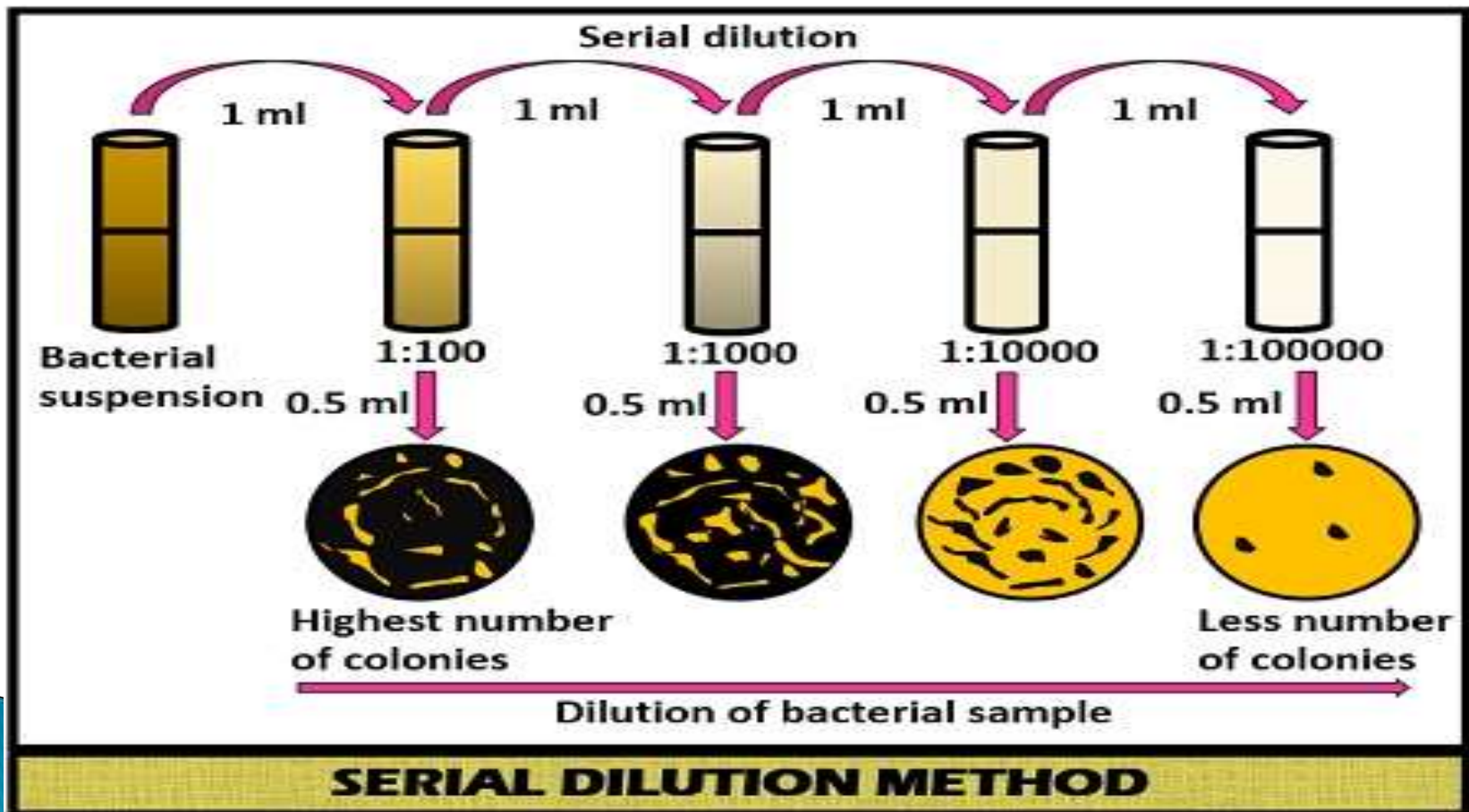
- ▶ **Streaking Method**
- ▶ Streaking method is very popular and the most widely used method for the isolation of pure culture. In this take the nutrient medium into the sterile Petri plates and allow it to solidify.
- ▶ After that take the inoculating loop and sterilize it on the flame until it gets red hot. Then, take the inoculum by the help of sterilized inoculating loop and streak over the solid nutrient media by keeping the plate close to the flame, to avoid contamination. After streaking of bacteria, incubate the culture plates for 24-48 hours at a temperature of 35-37degrees Celsius in the incubator.
- ▶ **Advantages-**

In the streaking method, there is a limited population of bacteria, from which the isolation of pure culture is quite easier than the pour plate and the spread plate method. In this, one can separate the individual colony of bacteria for the isolation and culturing of bacteria.

# Streak Plate Method



- ▶ Serial Dilution Method
- ▶ This technique is much known for the isolation and culturing of bacteria. In the serial dilution method, take the bacterial suspension and dilute it serially in successive test tubes.



- ▶ By following the “Serial dilution” method, add 1ml of the sample to the neighbouring test tube sequentially in a series  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$  and so on. After the sequential dilution of bacterial suspension, we can inoculate the bacterial culture by the above three methods, i.e. pouring, spreading and streaking.
- ▶ It is very easy to isolate bacteria from less number of a bacterial population. In serial dilution, the more concentrated sample, i.e.  $10^{-1}$  will produce the highest number of colonies. The more diluted sample, i.e.  $10^{-4}$  will produce the least number of colonies.
- ▶ So it should be clear to us that less diluted sample will contain more concentration of bacteria than that of water. And the more diluted sample will contain a high concentration of water than that of bacteria.

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