MEDICAL MICROBIOLOGY

LAB 4

Isolation of pure culture

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Pure culture

The identification process of an unknown microbe relies on obtaining a pure culture of that organism . A portion of an isolated colony then may be transferred to a sterile medium to start a pure culture . A microbial culture consisting of two or more species is said to be a mixed culture , whereas a pure culture contains only a single species .

To separate a colony from a group is called isolation , and there are several isolations techniques . The three methods for isolation are the **Streak plate method** , **Spread plate method** and **Pour plate method** , in all three methods , the purpose is to dilute the sample to obtain a pure culture.

Streak plate method

The loop is used for preparing a streak plate . This involves the progressive dilution of an inoculum of bacteria over the surface of solidified agar medium in a petri dish in such a way that colonies grow well separated from each other . The aim of the procedure is to obtain single isolated pure colonies .

Materials

1-Agar plates

2-Bacterial sample

3-Inoculating loop

4-Inocubator

Procedure

1-Preparation of an agar plate.

2-Sterilization of an inoculating loop.

3-Colling of the loop and takes a small amount of bacterial sample inoculated on the agar surface by marking parallel streak marks .

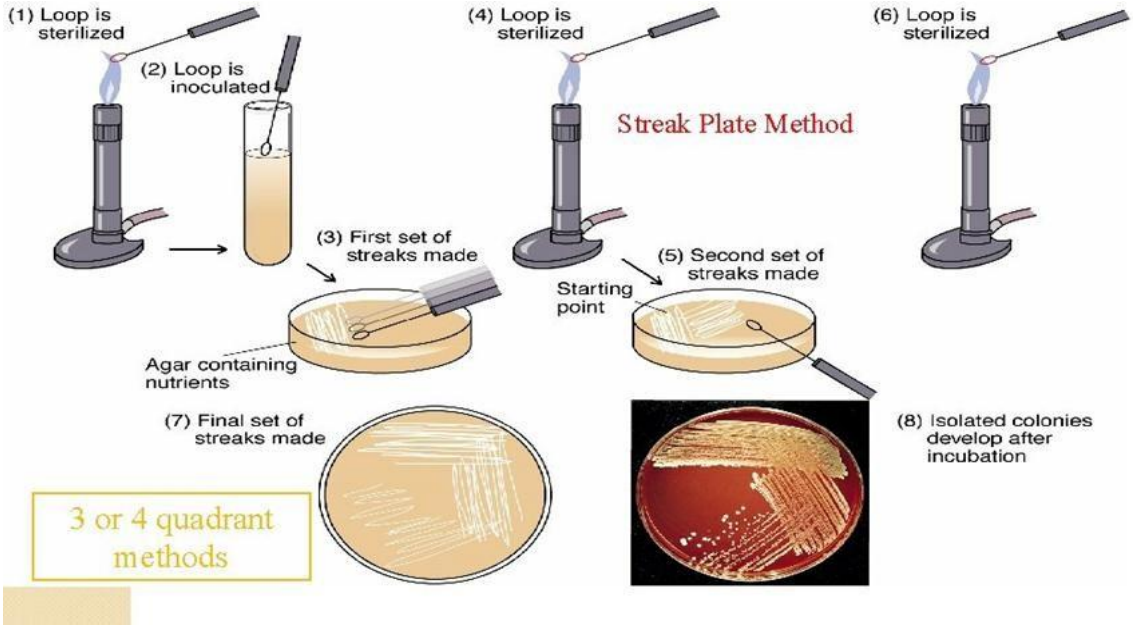
4-Loop sterilizes again and cooled and streaking continued from the end point of previous streak mark .

5- This process repeated several times .

6-Incubate the streak plates at 37^0C for 24 to 48 hours .

Observation

Bacterial growth could be observed along the streak marks and separated colonies on the later streak marks .



Spread plate method

This is a method by which bacteria are cultured on the surface of solidified agar medium . This method used for quantitative work (colony counts) and isolation of bacteria .

Materials

1-Agar plates

2-Bacterial sample

3-micropipette

4-L-shaped glass rod or plastic (spreader)

5-Bunsen burner

6-Inocubator

Procedure

1-Transfer of bacterial sample (0.1ml) on the agar surface in the petri dish.

2-Spread of bacterial sample with the L-shaped glass rod (spreader) by pushing it back and forth while rotating the agar plate .

3- Spreading continued until drying of the sample on the agar surface .

4-Inocubation the plates at 37 ^{0}C for 24 to 48 hours .

Observation

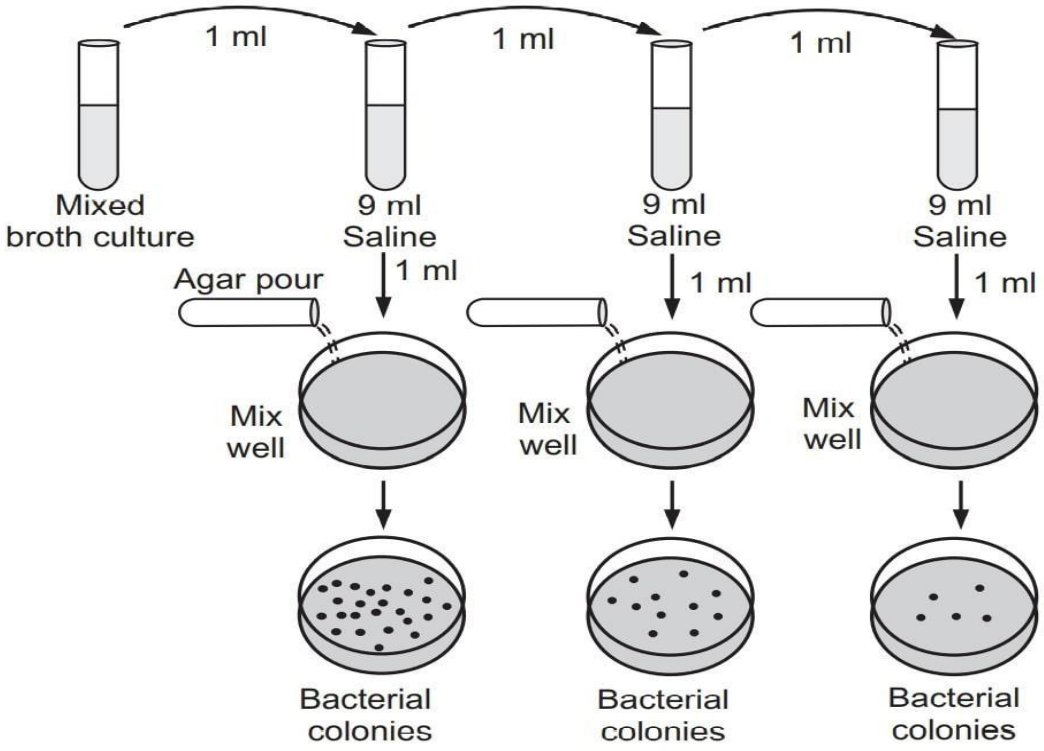
1-Clear and well separated bacterial colonies formed on the agar surface.

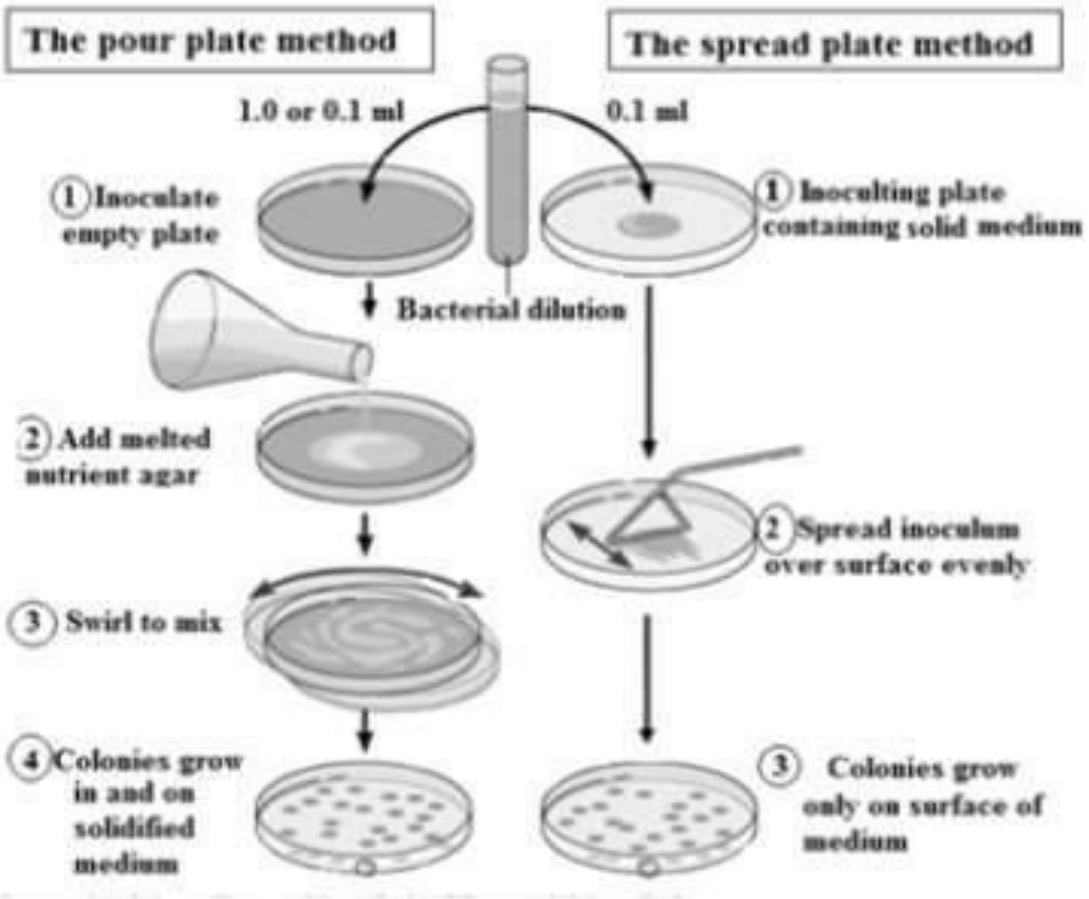
2-Colony colour and shape were observed .

Pour plate method

A pour plate is one in which a small amount of inoculum from broth culture is added by pipette to the centre of a petri dish , agar medium cooled and then poured into the petri dish containing the inoculum . The dish is gently rotated to ensure that the culture and medium are thoroughly mixed and the medium covers the plate evenly . Pour plates allow microorganisms to grow both on the surface and within the medium . The dilutions

chosen(1 cm^3) must be appropriate to produce between 30 and 100 separate countable colonies .




The pour plate method
The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
1 Inoculting plate containing solid medium
Bacterial dilution
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium