Lab-4 Practical Bacterial physiology

Measurement of Bacterial growth(direct count) viable count

Viable Count

A viable cell is one that is able to divide and form a visible colony on the nutrient media. Viable cells are counted by methods pour plate and spread plate.

Pour plate method

In this method, a known volume (0.1 or 1.0ml) of the culture is pipetted into a sterile petri plate, then molten nutrient medium is poured over and incubated. Colonies will appear throughout the agar medium and are counted to obtain viable count.

Spread plate method

In this method, a known volume of the culture (0.1ml) is plated and spread over solidified sterile agar medium, using a sterile spreader. The total number of colonies appearing on the plate after incubation represents the total number of viable cells in the culture.

Plating into the Media

The prepared samples can be plated to culture media via the pour plate or spread plate technique.

The procedure of the spread plate technique

1. **Pipet 0.1 mL of a diluted sample from T1 directly onto a petri dish labeled P1. Repeat this step with each tube and plate to the corresponding petri dish (T2 to P2, T3 to P3, and so on).**
2. **Obtain a sterile, disposable spreading rod or flame sterilize a glass spreading rod.**
3. **In a clockwise or counterclockwise motion, glide the horizontal portion of the spreading rod to equally distribute the sample through the petri dish's surface.**
4. **Incubate plates in a 37 incubator for 24 hours.**

Results and Analysis:

Colony Counting:

After incubation, the plates are examined for the presence of isolated colonies.

CFU Calculation:

The number of colony-forming units (CFU) is counted, and this number is multiplied by the dilution factor to determine the original microbial concentration in the sample.

Interpretation:

The morphology and characteristics of the colonies can provide information about the type of microorganisms present.

The procedure of the pour plate technique

. **Sample Preparation:**

- **A liquid sample containing microorganisms is prepared. Often, serial dilutions are made to ensure a countable number of colonies.**

2. **Agar Preparation:**

- **Agar, a solidifying agent, is melted and cooled to a temperature that is warm enough to remain liquid but not so hot as to kill the microorganisms (typically around 45-50°C).**

3. **Inoculation:**

- **A measured volume of the diluted sample (e.g., 1 ml) is pipetted into a sterile petri dish.**

4. **Pouring and Mixing:**

- **The molten agar is then poured over the sample in the petri dish.**
- **The dish is gently swirled to ensure the sample and agar are thoroughly mixed and evenly distributed.**

5. Solidification:

- **The agar-sample mixture is allowed to solidify at room temperature.**

6. Incubation:

- **The petri dish is inverted (lid on bottom) and incubated at an appropriate temperature and time for the microorganisms to grow.**

7. Colony Counting and Analysis:

- **After incubation, individual colonies will be visible both on the surface and within the solidified agar.**
- **These colonies can be counted to determine the colony-forming units (CFU) per ml of the original sample.**

Advantages of the pour plate method:

- **It allows for the isolation of microorganisms growing both on the surface and within the agar.**
- **It is suitable for isolating and enumerating bacteria, fungi, and other microorganisms.**
- **It is used in various applications, including food and pharmaceutical industries, soil microbiology, and research on microbial metabolis+**

Counting Number of Organisms

After 24 hours of incubation, remove the plate from the incubator and count the number of colonies using colony counter. The usual practice is to count colonies only on plates that have between 30 to 300 colonies.

Calculate the number of bacteria present in the original sample is calculated by multiplying the number of colonies formed with the dilution factor.

Notes

1. **Refrain from estimating colonies count by counting >300 colonies or <30 colonies on the agar plate, as it leads to a high degree of error. A high count can be confounded by the error in counting too many small colonies or difficulty in counting overlapping colonies.**
2. **Use separate sterile pipettes for each dilution. To do otherwise will increase the chances of inaccuracy because of the carry-over of organisms.**
3. **Accuracy in quantitation is determined by accurate pipette use and adequate agitation of dilution tubes.**

Pour Plate Method vs Spread Plate Method

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Pour Plate Method

1.0 or 0.1 mL

1. Inoculate empty plate

2. Add melted nutrient agar

3. Swirl to mix

4. Colonies can grow in and on the medium

Bacterial dilution

Spread Plate Method

0.1 mL

1. Inoculate plate containing solid medium

2. Spread inoculation over the surface evenly

3. Colonies can grow only on the surface of the medium

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BY: SELVAJOTHIPRASATH. G

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