Lab.4

Isolation of bacteria in pure culture

To identify a bacterial pathogens, it’s necessary to isolate the bacteria in pure culture, many techniques can be used to isolate different microorganisms.

1. Streak plate technique:
   Streak method is routinely employed for isolation of bacteria in pure culture. This technique involves the following steps:
   1. Put a sterile wire loop over the flame of the burner until be red-hot and allow to cool.
   2. Remove very small amount of bacterial culture or clinical materials by sterile wire loop.
   3. Hold the late of medium near to the burner by free hand and put the inoculums on peripheral of the plate and spread over a small area.
   4. Rotate the plate to 90° and streak over agar surface by making a parallel line vertical to the first one.
   5. Sterile the loop again and rotate the plate toward right angle and making another lines of streak vertical toward quarter by touching the previous lines.
   6. By the same process, repeat the streaking for 3 to 4 times until the whole surface of the agar plate is inoculated.
   7. Streak the center of plate in a zigzag motion.
   8. Incubate the plate in an inverted position at 37°C for 18-24hr.

2. Spread plate technique:
   In this method take 0.1ml of sample of bacterial culture by pipette place over the surface of agar medium. Spread the bacteria culture over the surface by glass spreader then incubate the plate at 37°C for 18-24hr.
   
   This technique is used for counting bacteria and also for swabbing from clinical lesions such as throat swab, ear swab, vaginal swab and others.

3. Pouring plate technique:
   1. Inoculate a sterilized empty Petri dish with 0.1ml of bacterial culture or sample by using sterile pipette.
   2. Pour the media into the Petri dish and allow to solidify.
   3. Incubate the plate at 37°C for 18-24hr.

   To differentiate between aerobic and anaerobic bacteria, aerobic bacteria grow and reach to the surface of the agar medium.
**Stabbing method:**
This technique is used to differentiate between motile and non-motile bacteria. The procedure steps include:

1. Put 10 ml of semi-solid media in a test tube and leave to cool in vertical position.
2. Inoculate with a straight wire (needle) making a single stab down of center of the tube to about half the depth of media.
3. Incubate in 37°C for 1-2 days.
4. Non motile bacteria give growth that is restricted to the line of the stab, while motile bacteria diffuse to media making a cloud of growth and the out-growth may reach of the wall of the media.

**Cultivation of anaerobic bacteria:**
Obligate anaerobic grow only in absent of O₂, because the bacteria lack mechanism of oxidation through respiratory enzymes (like cytochrome oxidase, catalase, peroxidase) resulting in H₂O₂ accumulation. The H₂O₂ is toxic for Bacterial growth.

The methods described for achieving anaerobic conditions are:

1. Exclusion of O₂ by heating or vacuum.
2. Absorption of O₂ by chemical means.
3. Reduction of O₂ by candle or Gas Pak Jar.

Gas Pak system is now the method of choice for isolation of anaerobes. It generate CO₂ and H₂ in moist condition. H₂ combine with O₂ to for H₂O in present palladium pellets, the pellets act as catalyst for combination.

Ethylene blue strip act as indicator, is colorless in anaerobic conditions, and remain blue in aerobically.

**The procedure:**

1. Place inoculated agar plates media into the Jar in an inverted.
2. Open the indicator strip and place it in the Jar.
3. Open the Gas Pak envelop and introduce 10ml D.W. into the envelop and put it in the Jar.
4. Place the lid and tighten the screw clamp.
5. Incubate the Jar at 37°C for 24-48 hr.