Cultivation

In order to identify bacterial species, we need to isolate bacteria in **pure culture** it is single species of microorganism (mass of bacterial cell derived from one mother cell)so it have the same origin and features. While in natural habitat, bacteria usually grow together in populations containing number of species (more than one type of bacteria at the same time) **mixed culture.**

Several techniques are employed to cultivate bacteria:

1- streak plate technique.
2-spread plate technique.
3-poure plate technique.

Primary culture : in which bacterial isolation and some of sample from it's original source are holed either by swab or loop ,then cultivate on ordinary media.

Sub culture : making another streak plate from the colony appeared in primary culture.

that mean when we need transferred microorganisms from one culture to another new one ,we make **sub culture**.

#the purpose of steak plate is to obtain isolated colonies by creating areas of increasing dilution on single plate .

Isolated (single) colony: clone cell originate from a single cell.

1-a- Method of making primary culture by streak plate technique:

1- label the bottom of the new Petri plate ; to be cultivated.
2-take small amount from sample (blood ,urine ,pus , sputum.....) and placed on the side of agar plate either by a swab or a sterile wire loop
3- sterile wire loop by flam until red hot , then cooled in the margin of the agar .
4-spread the sample from one side to another by streaking moving in a zig –zag horizontal on all the agar plate.
5-incubate the plate over night at 37 degrees Celsius.

1-b- Method of making sub culture to obtain pure culture and single colony by streaking plate technique:

1-lable the new agar plate.
2- sterile wire loop by flam until red hot , then cooled in the margin of the agar .
3-pick a colony and scrape off a little of the bacteria using the loop (from old agar).
4-streak the loop containing the bacteria at the top end of the new agar plate moving in a zig-zag horizontal pattern until 1/3 of the plate is covered.
5-sterilize the loop again in the flame and cool it at the margin of the new agar away from the bacteria.
6-rotate the plate about 60 degrees and spread the bacteria from the first streak into a second area using the same motion in step 4.
7-sterile the loop again using the procedure in step 5.
8--rotate the plate about 60 degrees and spread the bacteria from the second streak into a new area in the same pattern .
9- sterile the loop again.
10-incubate the plate over night at 37 C .

2-method of spread plate technique:

1-lable the new agar plate.
2- take one drop from bacterial culture(diluted liquid media) by micro pipette (100 ML) and placed in the center of agar plate .
3-immerse the L-shape glass rod into a beaker of ethanol and then tap the rod on the side of the beaker to remove any excess ethanol; briefly pass the ethanol-soaked spreader through the flam to burn off the alcohol and cool it in the margin agar.
4-spread the bacterial drop evenly over the agar surface by the sterile spreader making sure the entire surface of the agar has been covered.
5-immerse the spreader in ethanol using the procedure in step 3.
6-incubate the plate 24 hr at 37 C.

this method may be used for antibiotic sensitivity test .

3-method of poure plate technique:

1-lable the empty, sterile and new plate.
2-by using micropipette take amount of bacterial broth (0.1 – 1.0 ml) put it in the center of Petri dish .
3-cooled the melted agar media to 45C and add to the plate then mixed well genital swirling (the plat should be on the table).
4--incubate the plate 24 hr at 37 C.

#because the sample is mixed with molted agar medium a larger volume can be used than with the spread plate .

#bacteria then grow on top, inside and beneath the agar .

#this method is often used to bacterial enumerate after make serial dilution of bacterial broth and it can be supply a sufficient oxygen deficient environment .

Cultivation on broth media :

A-method of inoculation broth from solid medium:

1-lable new tube (contain sterile broth media).
2-sterile loop as we learn.
3-scrape the bacterial colony on agar media ,close the plate cover.
4-open the new tube ,immerse the loop in broth medium be sure close the tube.
5-sterile the loop.
6- incubate the tube at 37C over night.

B-method of inoculation broth from broth media :

1-lable the new tube .
2-using micropipette take (10- 100)ML from bacterial growth in old broth be sure close the cover.
3-transfer this growth to nev ı close the cover.
4-releas the tip of micropipette in trash .
5- incubate the tube at 37C over night.

Culture characteristics on solid media.

1-size: bacterial colonies have size from 5-10mm in diameter but genus *proteus* and *pseudomonas* spread on agar surface.
2-shap: circular, regular or irregular ,granular, filamentous,.....etc.
3-margin of edge: entire ,lobate , serrate , undulate,.....etc.
4-evelation: flat , convex, raised ,.....etc.
5-pigment: many of colonies have color like red, brown, violet, white, gray, while some colonies was colorless.
6-consistency: watery, viscous, membranous,..... etc.

Lecturer : Rawaa M. O. Hraishawi