LAB WORK 7.

Subject: Staining Microbial Structures: Capsule.
Session Purpose: Negative staining. Capsule staining.
Objectives:
1. Practice negative staining of bacteria.
2. Determine the capsule of *Azotobacter*.
2. Performexercises 1,2.

Capsules are mucilaginous substance, majorly composed of polysaccharides which are secreted by bacteria during their active growth and form a viscous coat around the cell. When this mucilaginous structure is irregularly arranged and loosely bound to the cell, referred by slime layer.

Capsule is partially synthesized in cytoplasm and composed of mainly polysaccharide but may contain other material like poly-D-glutamic acid. The ability of capsule formation is genetically determined. The capsule is well develop in some bacteria like *Azotobacter, Streptococcus pneumoniae, Clostridium perfringes* and *Klebsiella pneumoniae.*

To dertermine the presence or absence of capsule for classification and identification of bacteria is done by staining. There are two widely used methods for staining of bacterial capsule, which we will see below in detail: Capsule staining by negative staining methodand Anthony staining method.

Negative staining.

Simply flooding the smears with an appropriate basic dye is not always the best option to stain the microbial specimens. The fixation of smears results in shriveled or distorted cells which are not appropriate for measuring the size of the cells. Additionally presence of refractile bodies such as Endospores, storage granules and capsule forming microorganisms are not easy to stain by dyes hence it is devised not to stain the cells but the background. As in this modification the background is stained not the cells hence they appear transparent against the dark background and the process is referred as indirect staining. This is simplest and often quickest staining technique used for microorganisms.

The microbial cells contain various components in its outer layer (cell membrane, cell wall etc) which results in development of overall negative charge at surface of microbial cells. When a basic dye is applied, positive chromophore group get attached to the cell surface and provides colour to the cells. Indirect staining uses the just opposite mechanism which is the use of acidic dyes. Acidic dyes contain chromophores with negative charge hence repelled by the cell surface and causes darkening of the background against colorless cells. As this indirect method of staining uses acidic or negatively charged dyes, the technique is most commonly referred as negative staining. Nigrosin or India ink) is the most commonly used acidic dye for this purpose.

How negative staining is done?

Application of stain : The first step is to take a drop of nigrosin (a negative stain) and place at one end of a clean glass slide.

Inoculum transfer : After placement of stain, transfer a loop full of inoculum from a broth culture by inoculating loop on the applied stain. Then mix gently with the loop. If culture is taken from an agar medium then mix a drop of water in the nigrosin and try to emulsify without spreading the mix too much.

Spreading the smear: Take another clean slide and put it at 30-45 degree angle, on the mixed amount.When it starts to spread on the junction of slides swipe the slide to another corner forming a thin smear.

Drying the thin smear and observation: Allow the smear to air dry. After that apply a drop of oil-immersion on it and examine under 100X of microscope.

The technique of negative staining can be easily applied for:

- Getting quick informations about the cell shape, arrangement without any heat distortion.

- This technique is very useful to detect the presence of cell breakage and refractile inclusions in cells such as sulphur and poly-β-hydroxybutyrate granules and endospores without using any specific procedure.

- The most common and routine application of negative staining is in determining the size of the microbial cells using microbial technique.

Lab Exercise 1.Capsule staining by negative staining method.

Methodical instructions: In this technique bacterial background is stained byusing an acidic dye (e.g. nigrosin dyeor India ink), which carry a negative charge as a result is repel by bacterial cell that have also bears a negative charge as a result of which bacterial capsule that surrounded the cell is appears as clear zone between cell wall and dark background.

Procedure:

1. Apply the nigrosin dye or India inkon glass slide: Take a clean glass slide and put a drop of nigrosin dye close to one end of glass slide.
2. Apply the bacterial culture: Add a loopfuls of a broth culture into the drop of the stain and mix with the loop.
3. Prepare a thin smear: Place a edge of second slide at 30° angle on first slide and pushed away to other end of the slide to prepare thin smear of suspended organism.
4. Air dry the smear.
5. Observation the slide: Place a drop of oil-immersion on the slide and examine under 100x magnification.

Lab Exercise 2.Capsule staining by Anthony staining method.

Methodical instructions: This method is devised by **E.E. Anthony** in 1931 for capsule staining in bacteria. In this method two stains viz. crystal violet (1%aqueous) work as primary stain and copper sulphate act as a decolorizing agent as well as counter stain. Crystal violet color the both, cell wall as well as capsule and appears dark blue in color but capsule being non ionic will not absorb primary stain. On applying copper sulphate, excess stain removes out and capsular material become decolorized. The capsule finally appears light blue in contrast to the deep blue color of the cell. Procedureofthistechniqueis:

Procedure:

1.Prepare the smear: Take a loop full of microbial culture broth at one end of slide and prepare a heavy smear of the bacterium. Then allow the smear to dry.
2. Apply crystal violet: Flood the smear with crystal violet for 2minutes.
3. Wash off the dye: Wash off the dye with 20% copper sulphate.
4. Drain copper sulphate: Drain out the copper sulphate and gently blot dry the smear.
5. Observe it: Place a drop of oil-immersion on the slide and examine under 100 xmagnification.

Equipment:

- Microscope
- Slide
- Dropper bottle of water
- Disinfectant tray
- Culture of Bacteria in slant tubes
- Inoculation loop
- Burnerflame
- Nigrosin or India ink
- Crystal violet (1% aqueous)
- Copper sulphate20%
- Immersion oil