Lab 3

**Special Stain**

By which we can stain special structures of bacterial cell like spores, flagella and capsule.

**A-Capsule stain (negative stain):** Capsulated bacteria have the ability to

 form gelatinous materials outside the cell called capsule, many

 saprophyticus species can form capsule.

**Function of capsule:-**

* To protect the cell from desication.
* To protect the cell from harmful environmental conditions.
* Protect the cell from phacocytosis.
* Serve as a mean of adherance of organism to the surface.
* Appear to be help in holding the cell together after cell division, this leading to chain formation.
* It's possible that capsule may aid in blocking the surface of receptor of bacteria to bacteriophage.

**Chemical composition of capsule:-**

Capsul is a layer of gelatinous materials lies out side the cell wall of many bacteria, capsule is either poly saccharides e.g. *Streptococcus pneumoniae* or proteins (polypeptide) e.g. Bacillus anthracis or complex of hyaluronic acid and muco polysaccharide e.g*. Str. pyogenes*.

Procedure:-

1. By means of bacteriological loop, put a drop of nigrosin stain or india ink stain on a clean slide, then transfer a small amount of culture on the drop, mix and spread it .
2. Leave the preparation to dry and examine under oil immersion power



Note:- because capsule is highly hydrate polymer it will shrink dramatically when heat is applied.

**Results:-** clear zone cells of capsulated microorganism and the backing ground is black. (so we stain the surrounding of capsule and leave capsule unstained).



**B- Spore Stain:-**

Some species of bacteria form a special structure called (endospore) when the environment is not suitable such as: low of O2 , low of nutrient , change of PH and other conditions that prevent the growth of vegetative cell .

Spores are dormant forms , spore is unstained because it is impermeable to anything or to any stain.

**Spore consist of :-**

1. Core: consist of chromatin materials, proteins, lipids, enzymes, carbohydrates, minerals, less of water and greater of calcium .
2. Cortex: consist of muco peptide and cadipicolonate .
3. Coat: keratin like materials.

 The resistant of spore due to:-

 Low of metabolic activity.

 Low of enzymatic activity.

 Low amounts of water.

 High amount or concentration of cadipicolonate.

**We classify bacteria according to the presence of spore, location, and shape.**

 Sporulated bacteria

Presence

 Non sporulated bacteria

 Spherical

Shape

 Oval

 Centrally e.g. B. anthracis

Location Terminally e.g. Clostridium tetani

 Sub terminally e.g. Cl. botulinum

Procedure:-

1. Prepare a smear from young culture, fix with minimum heat.
2. Apply malachite green for 3-5 mins, heat the preparation untile steam rises, avoid boiling .
3. Wash with water .
4. Stain with carbol fuchsin for 1 min .
5. Wash with water .

![stainpic[1]]()

Result :- spore green, bacteria red



