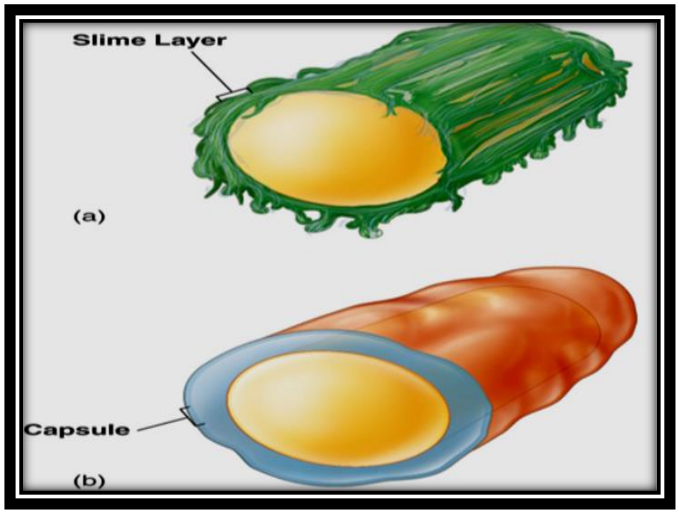
Microbiology lap.
8th lap.

Basma Bashar November - 2024

Capsule Staining (negative staining)

The bacterial cell is surrounded by a viscous gelatinous layer whose thickness varies according to the type. It may be a thin membrane in some species and is called a capsule, and a thick membrane in others.

If its structure is cohesive, its thickness may reach more than twice the cell. The capsule surrounds the individual cell or the chain of cells if the group is in chains. If its structure is disjointed, it is called a gel or a viscous layer (Slime). If its structure is solid, it is called the sheath.



The bacterial capsule is not stained in preparations stained by normal methods, as the cell appears surrounded by an unstained area, which is the envelope. Therefore, special methods are used to stain the capsule. In most cases, the capsule material is composed of complex, polysaccharide-rich carbohydrates. In some species, amino acids enter its composition.

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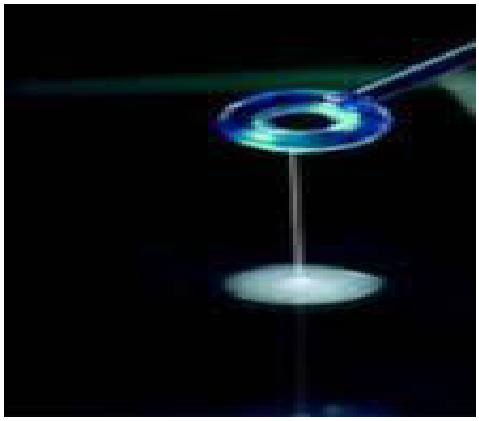
The importance of the capsule

Dryness, and ensuring resistance. The capsule protects bacteria from bad environmental conditions, especially against cellular phagocytosis, in addition to helping the cell adhere to surfaces, and the presence of the capsule has a clear effect on the appearance of bacterial cultures, and thus it is useful in distinguishing between species.

Capsules give colonies a mucoid glistening morphology which form a string when pulled with a loop as shown below:



- The main purpose of **capsule stain** is to distinguish capsular material from the bacterial cell. The capsule stain employs an acidic stain and a basic stain to detect capsule production. Negative staining methods contrast a darker colored, background with stained cells but an unstained capsule. The background is formed with India ink or nigrosine or Congo red. A positive capsule stain requires a mordant that precipitates the capsule. By counterstaining with dyes like crystal violet or methylene blue, bacterial cell

wall takes up the dye. Capsules appear colorless with stained cells against dark background.

- Procedure of Capsule Staining

1. Place a small drop of a negative stain (India Ink, Congo Red, Nigrosin) on the slide.

2. Using sterile technique, add a loop-full of bacterial culture to slide, smearing it in the dye.

3. Use the other slide to drag the ink-cell mixture into a thin film along the first slide and let stand for 5-7 min.

4. Allow to air dry (do not heat fix).

5. Flood the smear with crystal violet stain (this will stain the cells but not the capsules) for about 1 min. Drain the crystal violet by tilting the slide at 45-degree angle and let stain run off

until it air dries.

6. Examine the smear microscopically (100X) for the presence of encapsulated cells as indicated by clear zone surrounding the cells.

Capsule: Clear halos zone against dark backgroun