STAINING OF BACTERIA

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- Staining is a technique that is used for the examination of cells, tissues and cellular components. This is also the primary phase in the process of identification of an organism [stain is a reagent].
- To examine the bacterial cell, a variety of staining methods like simple, differential and special staining are used.

SIMPLE STAINING: In simple staining, the bacterial smear is stained with a single reagent, which produces a distinctive contrast between the organism and its background. Basic stains with a positively charged chromogen are preferred because bacterial nucleic acid and certain cell wall components carry a negative charge that strongly attracts and binds to the cationic chromogen.

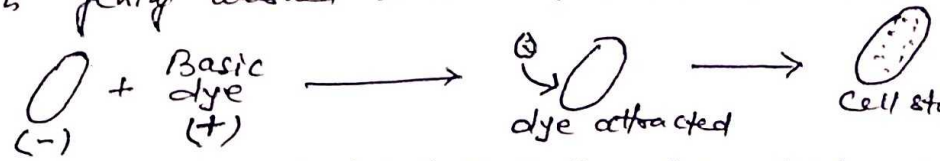
The purpose of simple staining is to elucidate the morphology and arrangement of bacterial cells. The most commonly used basic stains are methylene blue, crystal violet, and carbol fuchsin.

Procedure:

1. A small amount of bacteria is placed in a droplet of water on a glass slide. It is then air dried.
2. The slide is passed through a flame in a process called heat-fixing, which fixes the cells to the slides. The process kills most organisms and prepares them for staining.
3. Now the slide is flooded with a basic dye using appropriate exposure time for each:

   Carbol fuchsin – 15 to 30 seconds
   Crystal violet – 20 to 60 seconds
   Methylene blue – 1 to 2 minutes.

   The positively-charged dye is attracted to the bacterial cytoplasm which has a negative charge, and staining takes place.
4. The smear is gently washed with tap water to remove excess stain.



The slides are blot dried carefully and examined under oil immersion. This is effective for vegetative cells which appear purple

• Negative staining: In negative staining technique, an acidic, anionic dye is mixed with a bacterial cell sample. The dye changes the colour of the background, not the cells, causing the cells to stand out. This process is considered the opposite of simple staining.

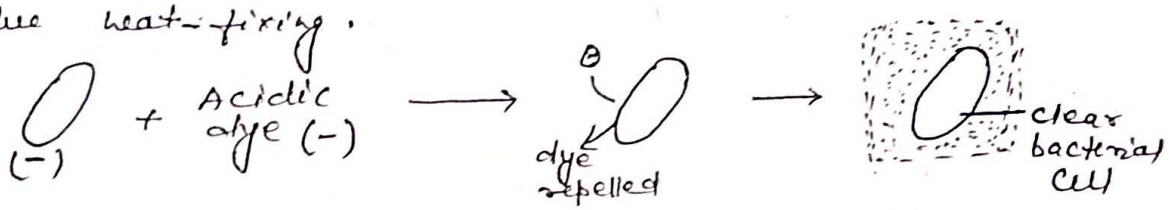
The principle of negative staining is that when the negatively charged dye is added to the negatively charged cells, the two repel each other, meaning they push apart. When the mixture is placed on a slide and air dried, a darkly dyed background surrounds clear, unstained cells. The transparent cells are clearly visible but are not affected by direct contact with the dye and distortion of heat fixing, which is not needed here.

Procedure: 1. Bacteria are mixed with an acidic dye such as congo red or black stains nigrosin on a slide.

2. The mixture is smeared across the face of the slide and allowed to dry in the air.

3. The stain gathers around the cell because of negative charges of both the organism and the dye.

The cells appear quite natural and larger than stained cells. The cells are not shriveled or distorted because it does involve heat-fixing.



Negative staining technique

DIFFERENTIAL STAINING: The differential stains use two or more stains and allows the cells to be categorised into various groups or types.

- Both the staining techniques (simple & differential) allows the observation of cell morphology, or shape, but differential staining usually provides more information about the characteristics of cell wall (thickness).
- MOST common and important differential stain is the GRAM STAIN. The other one is Acid fast stain.

Gram staining: This method of staining distinguishes bacterial species into two large groups – Gram-positive and Gram-negative bacteria. The name comes from the Danish bacteriologist Hans Christian Gram, who developed the technique in 1884.

Principle of Gram Stain: 1. The Gram stain technique is based on differential structure the cellular membranes and the cell walls of the two groups.

2. Gram-positive bacteria contain a highly cross-linked layer of peptidoglycan that retains the primary dye, crystal violet, following the application of mordant iodine.

The iodine and crystal violet form a complex within the peptidoglycan. when decoloriser is applied to the cells, the iodine-crystal violet complex remains within the cell, making it appear dark purple to blue.

3. The Gram-negative bacteria, which don't contain a thick cross-linked layer of peptidoglycan, do not retain the iodine-crystal violet complex following the application of decolorizer (acid-alcohol). The decolourizer dehydrates the outer layer, leaving holes in the membrane and effectively washing or removing the complex from the cells. The cells appear colourless. To make the colourless cell visible, a secondary stain, safranin, is applied leaving the Gram-negative cells pink.

Gram Stain Reagents:

Primary Stain : 2 g crystal violet, 20 ml 95% ethyl alcohol, 0.8 g ammonium oxalate and 100 ml. dist. water.

Gram's Iodine : 2 g potassium iodide, 1 g iodine crystals, and 100 ml. dist. water.

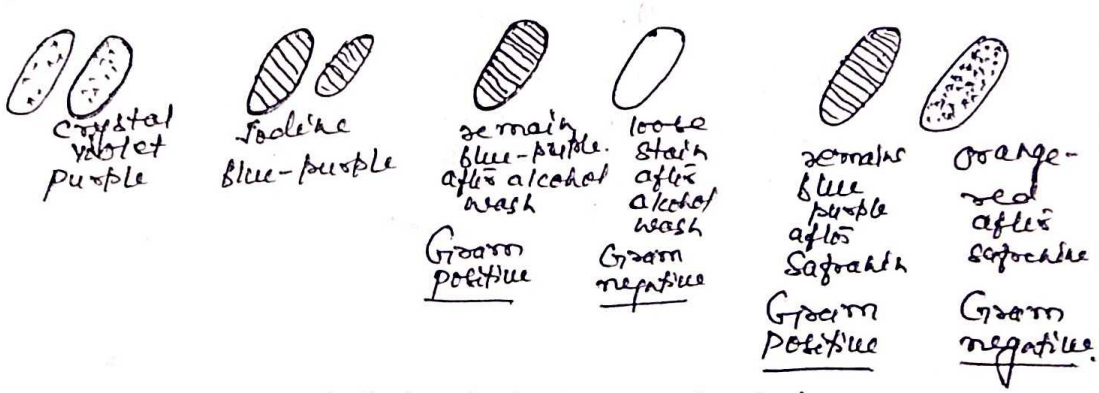
Decolorizer : 50 ml acetone and 50 ml ethanol / 95% ethanol

Counterstain : 4.0 g Safranin, 200 ml 95% ethanol, 800 ml. dist. water.

Procedure : 1. A thin smear of the bacterium is prepared on the slide.

2. To the smear crystal violet solution is applied for 30 seconds.
3. The slide is then gently rinsed in clean water, and the iodine solution is applied for 30 – 60 seconds. All the cells appear blue – purple at this stage.
4. The decolorizer is applied over the smear until all but the thickest parts of the smear have ceased to give off the dye. This usually takes from 20 to 60 seconds time.
5. The differential feature of the stain becomes now apparent. Microscopic examination will reveal that Gram-positive bacteria retain the violet-iodine combination (i.e. retaining of blue-purple colour even after alcohol wash, whereas Gram-negative ones loose the blue-purple colour after the alcohol wash and will be of original colour.
6. The counterstain such as safranin or eosin is applied. This colours the Gram-negative cells in orange-red.

In this way Gram positive and Gram negative bacteria can be differentiated.



Differential staining technique of bacteria.

Acid-fast Staining: 1. This technique is used to differentiate such bacteria which have waxy coating in their cell wall such as Mycobacterium.

2. These types of bacteria are difficult to stain by ordinary methods, and heat must be applied to force the dye molecules through to the cytoplasm.

3. Once stained, however, the cells are not easily decolorized, even with alcohol solution containing 5% acid. The organisms are said to be acid-resistant or acid-fast.

Procedure: 1. Air-dried heat-fixed smear is prepared.

2. The smear is stained with Ziehl-Neelsen's Carbolfuchsin while heating.

3. The cells become bright red.

4. The smear is then washed and rinsed with acid-alcohol decolorizer.

The acid-fast bacteria remain red while other bacteria loose their colour.

5. A counterstain methylene blue is then applied to decoloured bacteria. The slide is rinsed and dried for examination. The stain gives purple colour to the clear cells.

This technique is very useful to final diagnosis of tuberculosis bacterium. [A lipid detergent, tergitol is also added to carbolfuchsin, to dissolved the lipid in the cell wall and helps penetration of stain without heating].

SPECIAL STAIN TECHNIQUES:

Staining of Spores: 1. The spores, which are not stained by simple staining, can be stained by other means.

2. Heated malachite green stain is applied to a smear of bacteria.

3. The vigorous washing with water removes the stain from vegetative cells.

4. Safranin is then applied to the smear and washed gently.

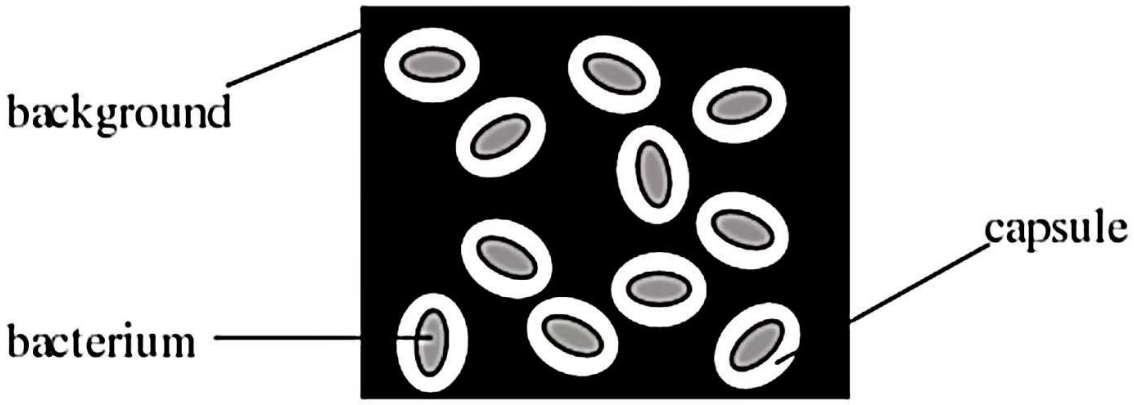
5. Spores take green colour and the vegetative cells red.

Staining of Capsule: Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to their surfaces. Therefore, the best way to visualize them is to stain the background using an acidic stain (eg. Nigrosine, Congo red) and to stain the cells using a basic stain (eg. Crystal violet, Safranin, basic fuchsin and methylene blue).

Procedure: 1. First a negative stain is done to outline the cell and its capsule.

2. Next a simple stain is applied to add colour to the bacterial cell.

The result is a stained bacterium cells with a colourless capsule on a clear background.



CONCLUSION: The various staining techniques [illegible] to study the morphology of bacteria, to differentiate different types of cells and also to study the different structures like spores and capsule. Depending upon the need and requirement, specific stain is used.

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