TUTORIAL 1 : GRAM STAINING

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OBJECTIVE

The objective of Gram staining is to stain bacteria as an aid to their identification which is either Gram-positive or Gram-negative. Gram-positive and Gram-negative is differentiated using Gram staining by their cell wall types. Gram-positive bacteria contain walls with relatively large amounts of peptidoglycan and no lipopolysaccharide while Gram-negative bacteria contain walls with small amounts of peptidoglycan. Other than Gram staining, bacteria can also be identified by shapes which are commonly spheres, rods, and spirals.

PRINCIPLES INVOLVED IN THE GRAM STAINING PROCEDURE

Gram-positive and Gram-negative bacteria are the two major groups of bacteria. Gram-positive and Gram-negative bacteria can be differentiated using the Gram Stain technique due to the differential structure of the cellular membranes and cell walls. The Gram staining technique uses crystal violet, iodine, alcohol, and safranin.

Crystal violet : Primary stain. It is used to stain the cell purple.

Iodine : Mordant. Makes the dye less soluble so it clings to cell walls.

Alcohol : Decolourizer. It washes away stains from Gram-negative bacteria cell walls.

Safranin : Counterstain. Allows dye to stick to Gram-negative bacteria cell walls.

1. A slide is put on a petri dish. 95% alcohol is poured and soaked for 30 seconds. Forceps is used to take out the slide. The slices are dried and heated by placing above the flame.
2. A loop of sterile distilled water is placed on the slide and a little bit of bacterial colony is put.
3. The slide is gently heated to fix the bacteria onto the slide.
4. The slide is placed on the staining rack. The smear is covered with a single drop of crystal violet for 30 seconds to one minute.
5. The slide is rinsed with slow running water gently.
6. The smear is covered with 2 drops of iodine. The slide is rotated and tilted to allow the iodine to drain. Cover again with iodine for 30 seconds to one minute. The procedure ensures that the iodine will be in contact with the cell walls of the bacteria on the slide since the iodine do no mix well with water.
7. The slide is rinsed with water as in step 6.
8. Several drops of 95% alcohol (decolouriser) are placed evenly over the smears, which is rotated and tilted with water. Alcohol is added until most of the excess stain is removed and the alcohol running from the slide appears clear.
9. A few drops of safranin are added on the bacterial smear and is left for approximately 30-45 seconds.
10. The smear is rinsed off with water and blot dry with filter paper.
11. The slide is observed under oil immersion magnification and the observation in terms of bacteria, shape, colour and whether it is Gram-positive or Gram-negative is described.
12. Steps 2-11 are repeated for microorganisms found in yoghurt.

LIMITATIONS ON GRAM STAINING

Gram staining is a technique which requires us to follow its procedures precisely in order to get an accurate result. Due to this, there are many limitations present in Gram staining.

Mis-identifying Gram-positives as Gram-negatives and Gram-negatives as Gram-positive is a common error in Gram-staining. For example, Gram-negatives can appear as Gram-positives due to the smear being too thick. This results in Gram-negatives not being fully decolorised during the decolourisation procedure and appearing as Gram-positive bacteria.

Gram-positive bacteria can also lose its ability to retain crystal violet and stain Gram negatively. This is due to its cell wall being damaged. This is usually due to antibiotic therapy or excessive heat fixation of the smear or the use of a very old Iodine solution.

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