

B.Sc. III Year (Theory)
Semester –VI
Paper XX (C)
Microbiology and Disease Management

Unit-1
C. Staining

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C) STAINING IN PLANT PATHOLOGY

1) Simple stain technique: A small amount of bacteria is placed in a drop of water on a glass slide, and then air dried it. The slide is passed through a flame in a process called heat fixing which fixes the slides, kills most organisms and prepares them for staining. Now the slide is flooded with a basic dye such as crystal violet or methylene blue for a minute. The positive charged dye is attracted to the bacterial cytoplasm which has a negative charge and staining takes place. This is effective for vegetative cells, the stain do not easily penetrate spores.

2) Negative stain technique: It is opposite to simple stain technique. Bacteria are mixed on a slide with an acidic dye such as Congo red or black stain nigrosin. The mixture is smeared across the face of the slide and allowed to air dry, because the stain carries negative charge. The stain gathers around the cell, since a chemical reaction has not takes place, heat fixing has been avoided, and the cell appear less shriveled or distorted. They often appear large than stained cells and more natural.

3) Gram stain technique:

A Danish scholar **Christian Gram in 1884** devised a differential staining procedure, which differentiate between two kinds of bacteria Gram positive and Gram negative bacteria. The procedure is called Gram staining technique.

1. A thin smear of bacteria is prepared on the slide.
2. To the smear crystal violet solution is applied for 30 seconds.
3. The slide is gently rinsed in clean water and iodine solution is applied for 30 seconds.
4. This in turn rinsed off.

5. If the slide is examined, all cells would be deeply stained and appear blue purple.
6. Then 95% ethyl alcohol is applied and this is renewed until all but the thickest parts of the smear have ceased to give off the dye.
7. This usually takes from 20 seconds to one minute.
8. Microscopic examination of the slide will reveal that Gram positive bacteria retain the violet iodine combination (retaining and, blue-purple colour after alcohol wash).

Whereas Gram negative ones loose the blue purple colour after alcohol wash. Those species retain the stain are called Gram positive whereas those which yield the stain to alcohol are called Gram negative bacteria.

Then there is applied counter stain a dye of some contrasting colour i.e. eosin (red), saffranin (red), light green. Exact of these colours the Gram negative species the cells become clearly visible. It has been postulated that since Gram negative bacteria have relatively high lipid content in their cell walls, the alcohol dissolve the lipid that allows the leakage of crystal violet-iodine complex. The Gram positive bacteria with less lipid in their cell wall, less susceptible to the action of alcohol.

1) Cotton Blue:

Aniline Blue	0.1 gm
Phenol	25 gm
Glycerine	25 cc
Lactic acid	25 cc
Distilled water	25 cc

This stain is used for staining various fungi.

2) Lactophenol:

This mounting media can be prepared by adding equal quantity of phenol, lactic acid, glycerine and distilled water.

Phenol	25gm
Lactic acid	25 cc
Glycerine	25 cc
Distilled water	25 cc

This medium is used for staining of various fungi.

Thank You