Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY LABORATORY

EXPERIMENT # 3

AIM

To study morphology of bacterial organisms by simple staining and to perform Gram staining of the given bacterial cultures.

BACKGROUND

Simple staining of bacterial organisms is very important starting point for identifying species. In this procedure, single stain is used to colour a bacterial organism. Most commonly used dye for simple staining is methylene blue, crystal violet, and basic fuchsin. These dyes contain colour-bearing ions (chromophores) and are positively charged (cationic). As bacteria are slightly negatively charged, they produce attraction with cationic chromophores of dyes called basic dyes.

Gram staining method is named after a Danish bacteriologist Hans Christian Gram (1882). It is one of the most important staining techniques in Microbiology and is usually the first step in identification of unknown culture. It is also used for the classification of bacteria into two general groups, namely, Gram positive and Gram negative.

MATERIALS REQUIRED

Microbial cultures, stain – methylene blue, basic fushein and crystal violet, ethanol, iodine, safarnin, distilled water, tissue paper, oil immersion, grease-free slides, microscope, etc

PROCEDURE FOR SIMPLE STAINING

1) Prepare a smear of culture on the microscopic slide and air dry.
2) Heat-fix cells by passing the slide rapidly across the flame (do not overheat as it may shrink the cells and distort the shape)
3) Flood the slide with methylene blue and allow it to react for 1 minute.
4) Tilt the slide and wash with distilled water to remove excess stain (be gentle).
5) Blot off excess water and allow it to dry completely.
6) Carefully position the oil immersion objective over the smear after adding immersion oil to the smear.

PROCEDURE FOR GRAM STAINING

1) Prepare a smear of culture on the microscopic slide and air dry.
2) Heat-fix cells by passing the slide rapidly across the flame (do not overheat as it may shrink the cells and distort the shape)
3) Flood the slide with crystal violet and allow it to react for 1 minute.
4) Tilt the slide and wash with distilled water to remove excess stain (be gentle).
5) Flood the slide with Gram's iodine and allow reacting for 1 minute.
6) Tilt the slide and wash with distilled water to remove excess stain.
7) Wash the smear with alcohol for 15-20 sec, by holding the slide in vertical position and observing the release of CV-I complex as blue drops. (This step is done till blue drops stop appearing).
8) Wash the smear with distilled water.
9) Flood the smear for one minute with safaranin.
10) Tilt the slide and wash with distilled water to remove excess stain (be gentle).
11) Blot off excess water and allow it to dry completely.
12) Carefully position the oil immersion objective over the smear after adding immersion oil to the smear.