

Indian Institute of Technology Delhi
Department of Biochemical Engineering and Biotechnology

I SEMESTER
BBL132 – GENERAL MICROBIOLOGY
LABORATORY

EXPERIMENT # 5

AIM

To study bacterial spores by staining methods.

BACKGROUND

Certain species of bacteria (Genera: *Bacillus*, *Clostridium*) in order to protect themselves from the vagary of adverse environment develops on its surface thick tough spore coat. **Endospores** are metabolically inactive, highly resistant structures produced by some bacteria as a defensive strategy against unfavorable environmental conditions like heat, short wave, radiation and toxic chemicals. Endospores can remain dormant for long periods of time. Spore coat is made up of protein dipicolinate. Simple staining will not stain endospores, however special stains and heating can dye the endospore coat. The endospore staining is a **differential staining method** which selectively stains bacterial endospores.

Schaeffer-Fulton Method - Malachite Green is a stain that is used to stain spores. It is triarylmethane dye with basic N-N- Dimethylaniline groups that interact with the thick & highly cross-linked surface of endospores.

MATERIALS REQUIRED

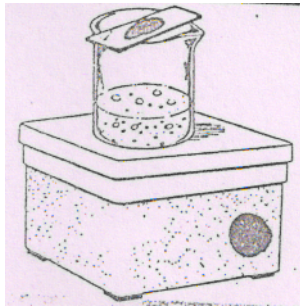
Culture : *Bacillus sp.*
Stains : 5% Malachite Green and Safranin
Apparatus : Slides, boiling water bath, pipettes, microscope, immersion oil.

PROCEDURE

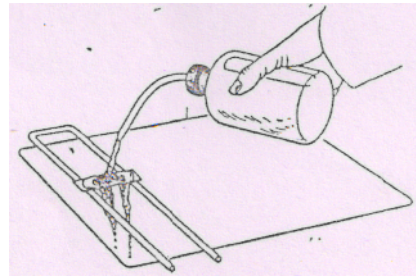
- 1) Prepare a thin bacterial smear on a clean grease free slide.
- 2) Air dry and heat-fix the smear.
- 3) Overlay the smear over a boiling water bath so that it is constantly exposed to steam.
- 4) Cover the smear with a sheet of blotting paper.
- 5) Flood the smear with malachite green stain for a period up to 20 min and place a cut piece of paper towel over the smear (Don't let the smear dry; keep adding stain to keep it wet).
- 6) Gently heat the under side of the slide with a burner by passing back and forth (Steam heating)
- 7) Keep the stain steaming for 15 min; add stain to keep the paper saturated.
- 8) Remove the paper towel and wash the slides with distilled water.

- 9) Counter stain with safranin for 1 min, wash and air dry.
10) Examine under oil-immersion objective. See spherical green endospores and pink vegetative portion of the cell (Sporangium).

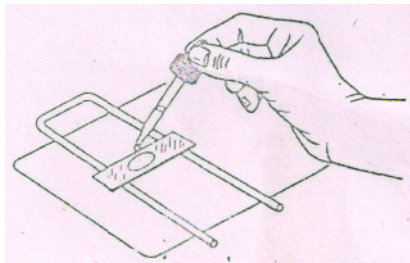
**1. Cover the smear with malachite green.
Steam over boiling water for 15 min.
Add additional stain if stain boils off.**



**2. After the slide has cooled sufficiently,
rinse with water for 30 seconds**



3. Counter stain with safranin



4. Rinse with water

