Endospore Stain

Objective/ purpose/ learning goals:

After performing this lab exercise, students will be able to

a. explain why endospores are produced by bacteria
b. perform endospore staining procedure
c. differentiate bacterial spores from vegetative cells on their appearance under microscope

Key terms

Malachite green, safranin, endospore, vegetative cell

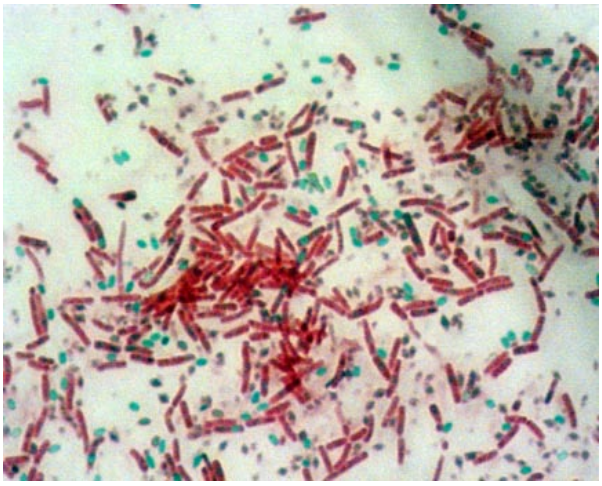
Introduction / theory/overview:

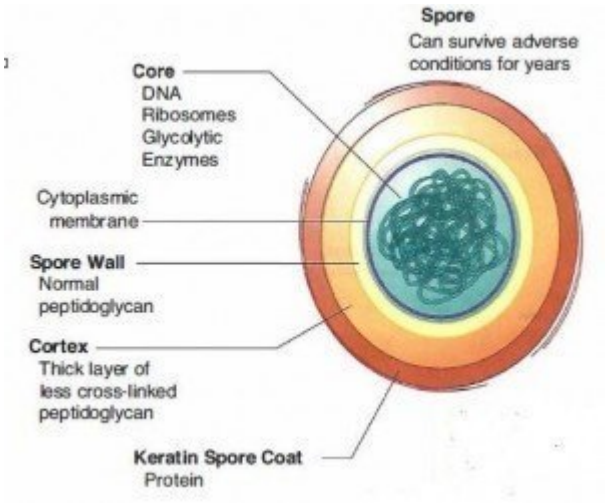
In response to adverse environmental conditions, like nutrient shortage, some bacteria produce highly resistant, dormant structures called spores or endospores. Spores do not have a role in reproduction. They help in the survival of the organisms during adverse environmental conditions. **Mature endospores** are released from the vegetative cell to become **free spores.** Spores can survive for many years in soil and other non-living objects. When the free spores find an environment that supports growth, they germinate to a vegetative cell.

An endospore is highly resistant to heat, disinfectants, radiation and dehydration. It is structurally and chemically more complex compared to the vegetative cells. It contains more layers than the vegetative cell. It has a **core** that includes a complete bacterial genome, a few ribosomes and glycolytic enzymes suspended in a small amount of cytoplasm; a **cytoplasmic membrane** that encloses the core; a **spore wall** made of normal peptidoglycan; a **cortex** made of less cross-linked peptidoglycan; and a **tough outer coat** made of the protein keratin.

The shape and the position of spores within the vegetative cell vary in different species and can be useful for classification and identification purposes. Endospores may be located in the **middle** of the cell (**central**), at the **end** of the cell (**terminal**) or near the end of the cell (**subterminal**). The shape of the spore can be spherical or elliptical.

Two **clinically important** genera of Gram-positive rods, *Bacillus and Clostridium*, produce endospores.





Principle of Spore Staining:

A differential staining technique (the Schaeffer-Fulton method) is used to distinguish between the vegetative cells and the endospores. Malachite Green is used as the primary dye to stain endospores. Malachite green permeates through the endospore wall as heat is applied (heating acts as a mordant in this case). Special decolorizing agent is not necessary in spore staining as the primary dye malachite green is water-soluble and does not adhere well to the cell wall. It washes off easily from the cell wall. The vegetative cells have been disrupted by heat. Therefore, malachite green also rinses off easily from the vegetative cells. However, the dye that is permeated through the spore wall is locked in and cannot escape. Safranin is used as a secondary/counter stain to stain the decolorized vegetative cells.

When visualized under microscopy the cells should have three characteristics:

1. the vegetative cells should appear pink/red (color of safranin),
2. the vegetative cells containing endospores stain pink while the spores within the cells should appear as green.
3. mature, free endospores that are released from the cells should not be associated with the vegetative bacteria and should appear green as well (note their size and shape). Free spores can be mistaken as cocci.

Materials, equipment and organisms (per student/ per group):

a. Live Organisms: *Bacillus subtilis or Bacillus cereus*, soil plates
b. Two slides per student for smears
c. Standard materials used for making, drying and heat-fixing smears,
d. Staining hot plate, **malachite green dye, and filter paper.**

Procedure:

a. Obtain a slide and make two smears, one for ***Bacillus sp.*** and one for the soil plate on the same slide. Follow the procedure for drying and heat-fixing the smears. Each student will make at least two slides, each with smears for both organisms side by side.
b. Find the fume hood where the spore staining materials are located
c. Put heat fixed smears onto staining hot plate in the fume hood.
d. Place filter paper(s) on the heat fixed smears and use the dropper to completely saturate the filter paper with **malachite green** dye.
e. Allow dye to stain your smear for at least 5 minutes.
f. Wipe off the bottom of the slide with a wet paper towel and decolorize by rinsing off the smear with tap water.
g. Counterstain with safranin for a minute.
h. Rinse off the counter stain, blot dry (and place coverslip if required by your instructor).
i. View your slide at 100X under oil
j. You may wipe the **BOTTOM** of the slide with lens cleaner to remove excess malachite green that has dried on the bottom. **NEVER** wipe the top of the smear!

Safety and disposal:

Follow standard lab safety procedure. Dispose of slides into the glass disposal container, as the organisms are heat-fixed and killed.

Observations/ interpretation/ Results:

Record your observations below:

Take home Lesson:

You need to know the reagents and their function in the staining procedure. Why do we use the endospore stain? For what organisms do you expect to find spores? What function does an endospore have?