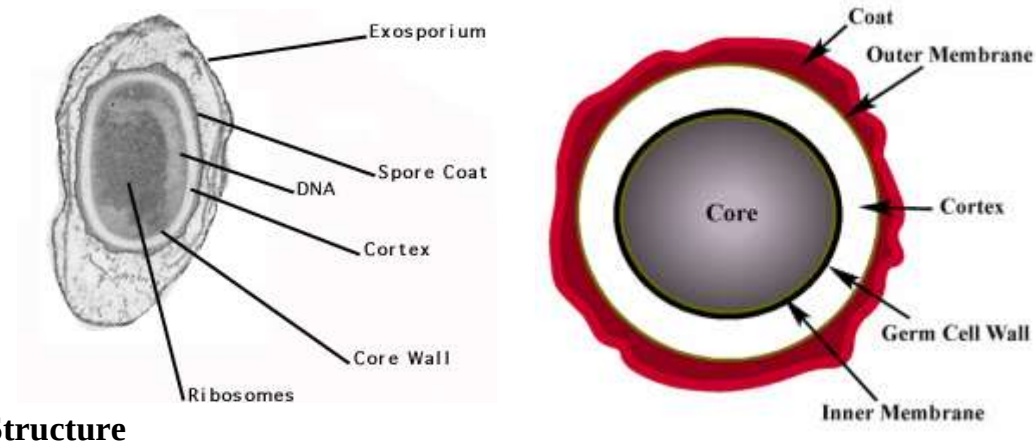
Structural Staining Method
(Endospore staining)

Vegetative cells are bacteria that are actively growing, metabolizing and dividing. When they are subjected to environmental stresses such as high temperature, high UV radiation, desiccation, chemical damage and enzymatic destruction, they eventually die. However, some bacteria can circumvent the problems by forming endospores. Endospores are metabolically inactive forms of a bacterium that allow it to survive the harsh environmental conditions, because they are comprised of a tough proteinaceous covering called keratin.

A mature endospore contains a complete set of the genetic material, ribosomes and specialized enzymes. Mature endospores are released from the vegetative cell and placed in an environment that supports growth they will revert back to a vegetative cell in a process called germination. It should be noted that endospore formation is not a reproductive process but is a mechanism for survival. Most endospore forming bacteria are found in soil or aquatic environments. However, some species of *Bacillus* and *Clostridium* have medical significance.

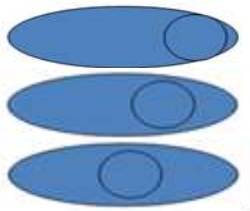


Endospore Structure

The exosporium coat is the outermost glycoprotein layer of the spore. This composition gives hydrophobic characteristic that makes it insoluble and contributes to the pathogenicity of spores. **The outer proteinaceous coat** lies under the exosporium and surrounding the spore provides much of the chemical and enzymatic resistance. Beneath the coat resides a very thick layer of specialized peptidoglycan called the **cortex**. Proper cortex formation is needed for dehydration of the spore core, which aids in resistance to high temperature. **A germ cell wall** resides under the cortex. This layer of peptidoglycan will become the cell wall of the bacterium after the endospore germinates. **The inner membrane**, under the germ cell wall, is a major permeability barrier against several potentially damaging chemicals. The center of the endospore, **the core**, exists in a very dehydrated state and houses the cell's DNA, ribosomes and large amounts of dipicolinic acid.

Endospore Location

1. Terminal as in *Clostridium tetani*.
2. Subterminal as in *Clostridium botulinum*.
3. Central as in *Bacillus anthracis*.



How is Endospores Formed?

When a vegetative cell of an endospore-forming bacteria detects that essential nutrients are running out it begins to sporulate, a process that takes about 8-10 hours and results in the formation of one endospore.

Endospore staining:

Is a technique used to identify the presence of endospores in bacteria, which can be useful for classifying bacteria. Endospore is difficult to stain using normal techniques. Special techniques for endospore staining include the Schaeffer-Fulton stain and the Moeller stain.

A differential staining technique (the Schaeffer-Fulton method) is used to distinguish between the vegetative cells and the endospores. A primary stain (malachite green) is used to stain the endospores. Because endospores have a keratin covering and resist staining, the malachite green will be forced into the endospores by heating. In this technique heating acts as a mordant. Water is used to decolorize the cells; the endospores will retain the primary dye while the vegetative cells will lose the stain. The addition of a secondary stain (safranin) is used to stain the decolorized vegetative cells.

When visualized under microscopy, the vegetative cells that contain endospores should stain pink while the spores should be seen as green ellipses within the cells.

Procedure (Schaeffer-Fulton Method)

1. Perform a **bacterial smear** of *Bacillus* sp.
2. Place a small piece of paper over the smear. Saturate the paper with **malachite green**.
3. Heat the slide gently over the Bunsen burner (**steamer**) for **5 minutes**. Be sure to keep the paper saturated with malachite green during heating. If the slide is steaming, you're okay; if it stops steaming, add more malachite green!
4. Remove the paper from the slide, and rinse the slide gently with water. And let's the slide dry.
5. Counterstain with **safranin** for **2 minutes**.
6. Rinse the slide gently with water. Dry the slide.
7. Observe the slide using the oil immersion microscope.