222 MBIO
Microbial Fine Structure

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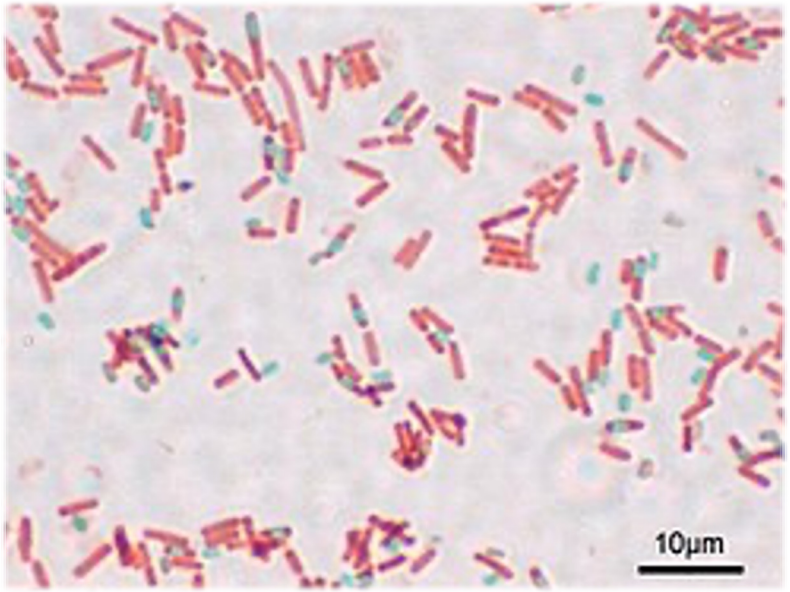
Bacterial Endospres

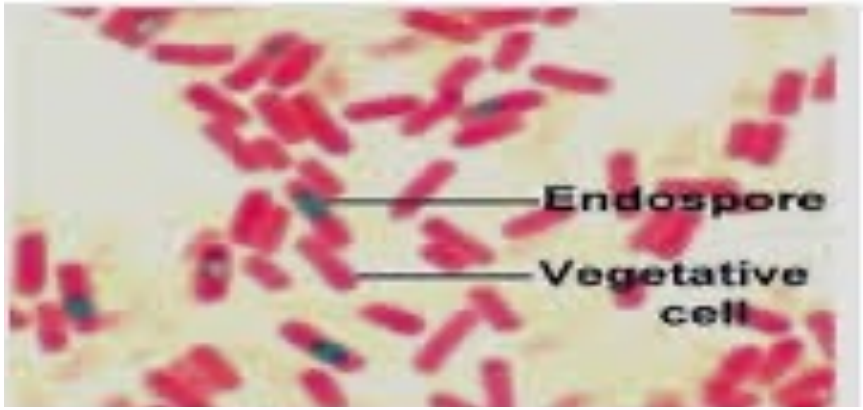
- Lab 7 -

Definition and Characteristics

- Endospores are **dormant (inactive)**, highly resistant structures formed by certain bacteria.
- They are typically formed by **Gram-positive bacteria**, such as ***Bacillus*** and ***Clostridium***.



- **Sporulation:** This process occurs in response to **nutrient deprivation** or **environmental stresses**.
- **Reactivation:** When the environment becomes **favorable**, the endospore can reactivate itself into a **vegetative state**.
- **Function:** Endospores are **protective structures** rather than **reproductive structures.**

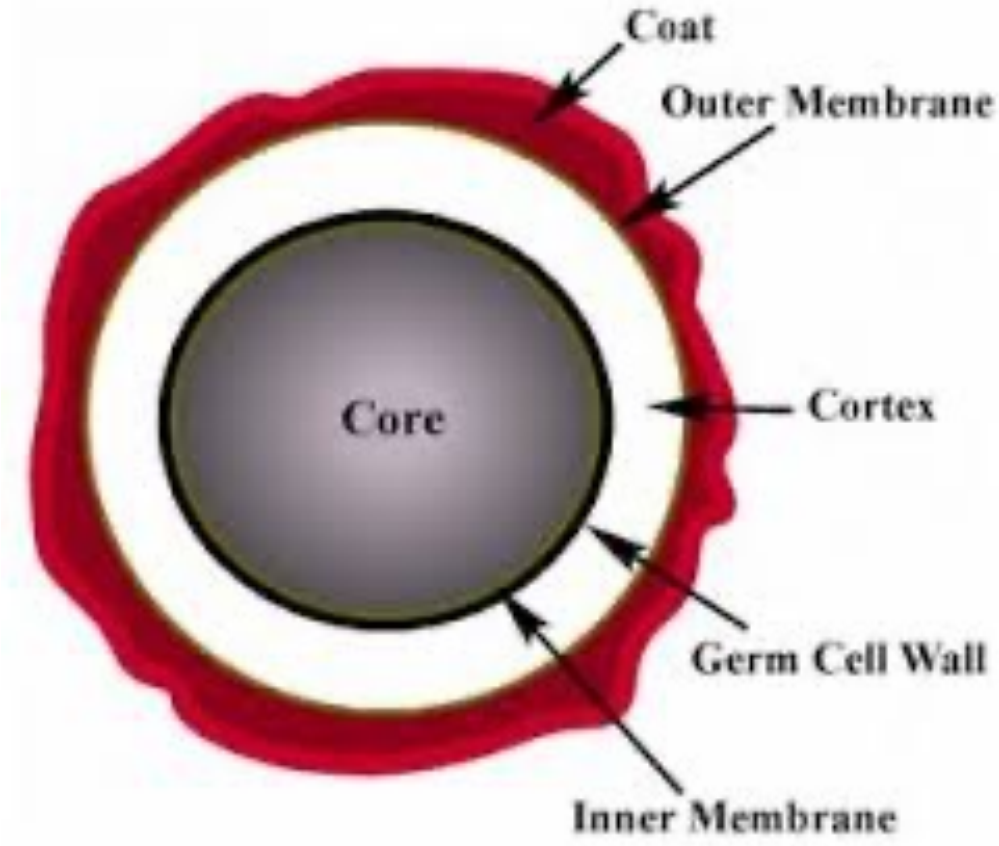


Structure of Endospores

- Endospores possess a complex, multi-layered structure:

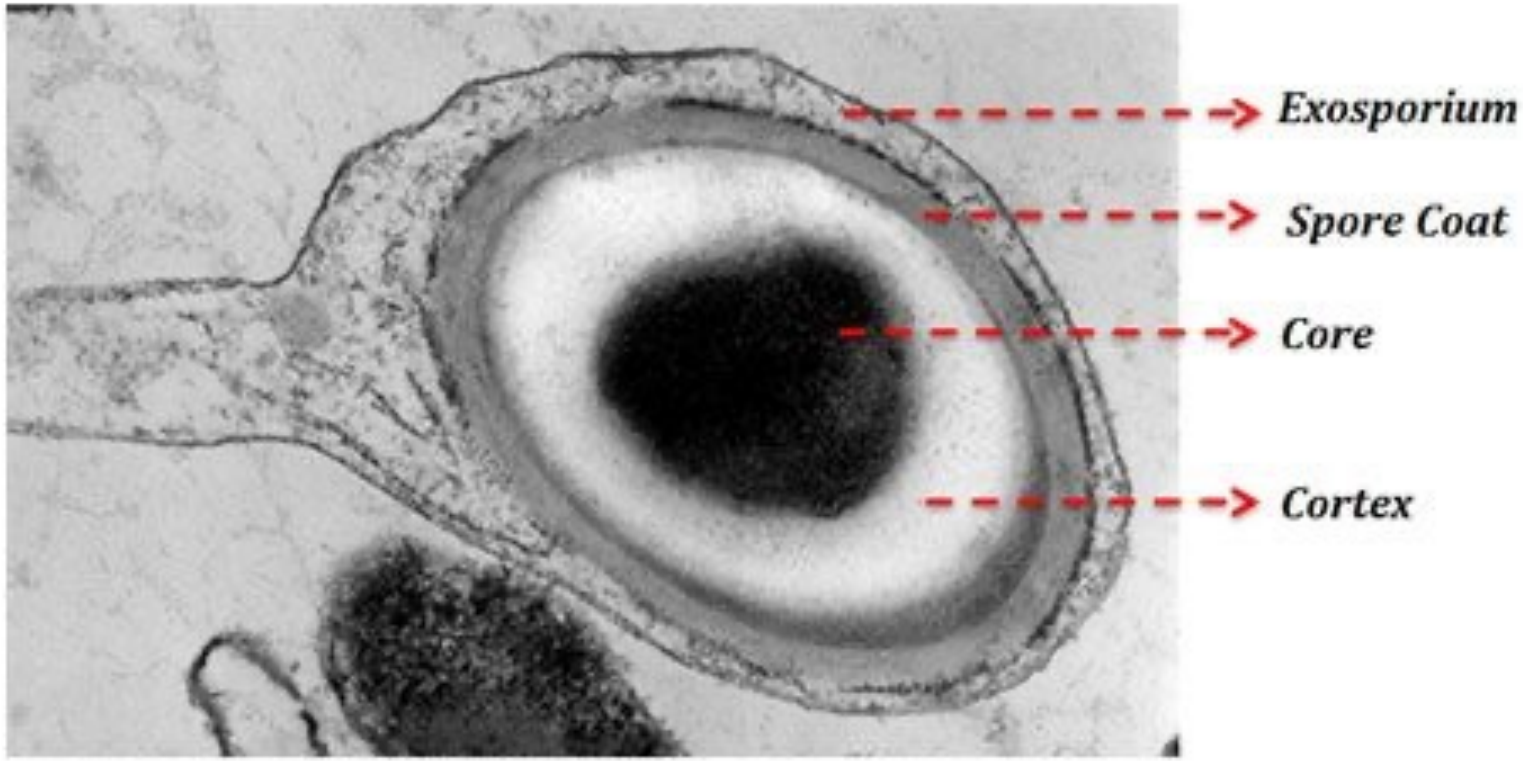
1. **Core:** Contains the **bacterium's DNA**, **ribosomes**, and **essential enzymes** for germination. It is in a **dehydrated state** to maintain dormancy.

2. **Cortex:** A **thick layer of peptidoglycan** that provides structural support.



3. **Spore Coat:** **A tough protein layer** that protects the core from heat and chemicals.

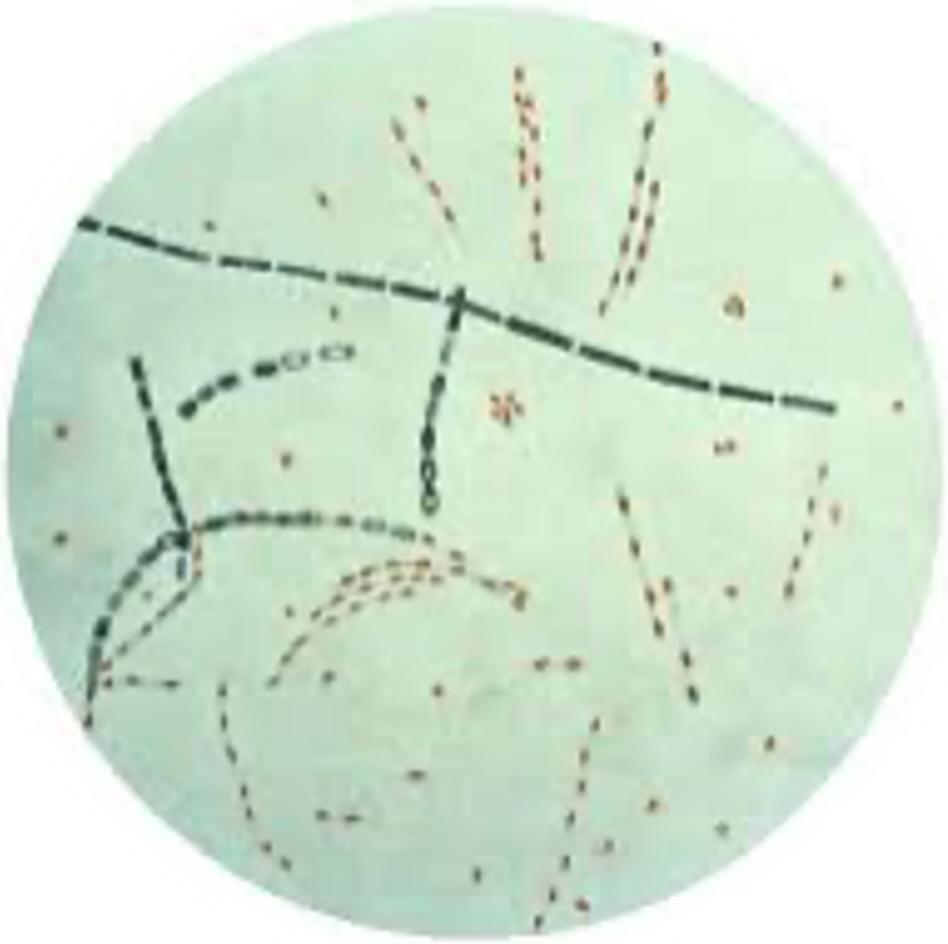
4. **Exosporium:** An **optional outer layer** providing **additional protection**.



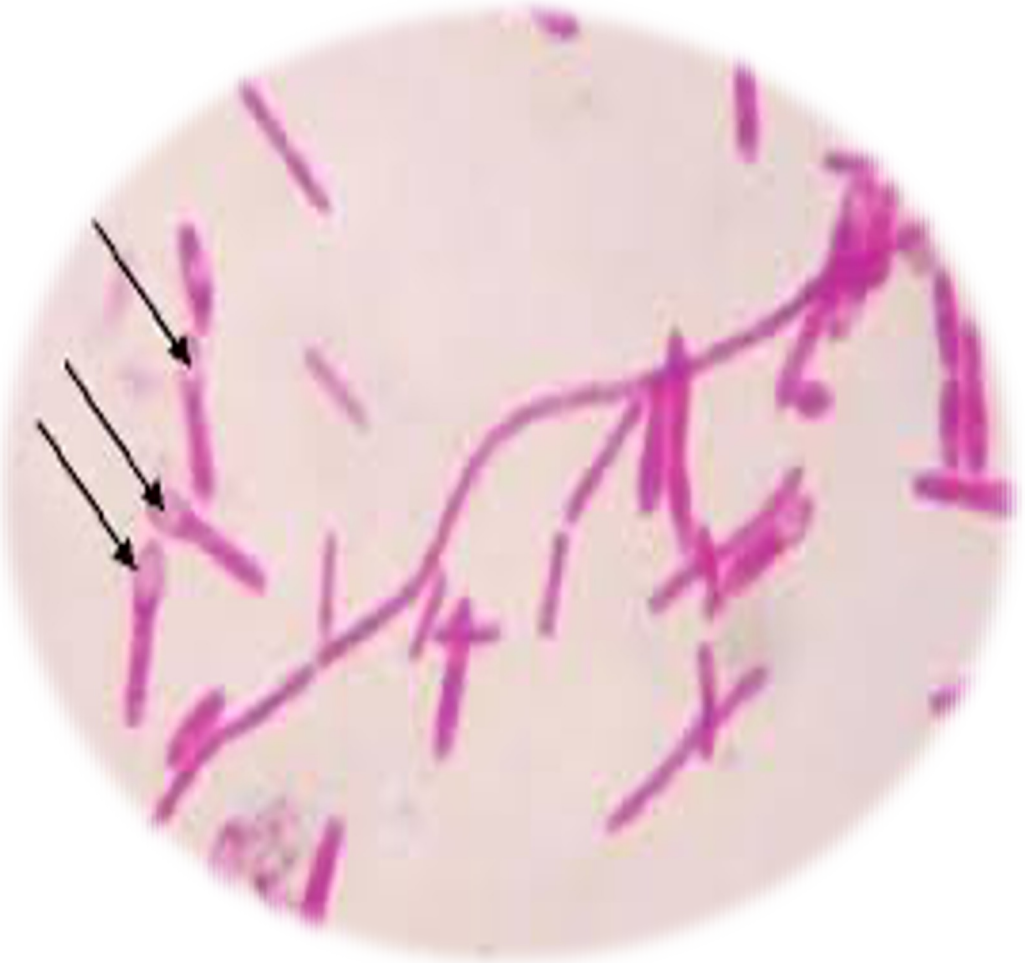
Medical Importance

- Several spore-forming bacteria are responsible for serious diseases:

Bacteria	Disease
Bacillus anthracis	**Anthrax**
Clostridium botulinum	**Botulism**
Clostridium perfringens	**Gas gangrene**
Clostridium tetani	**Tetanus**

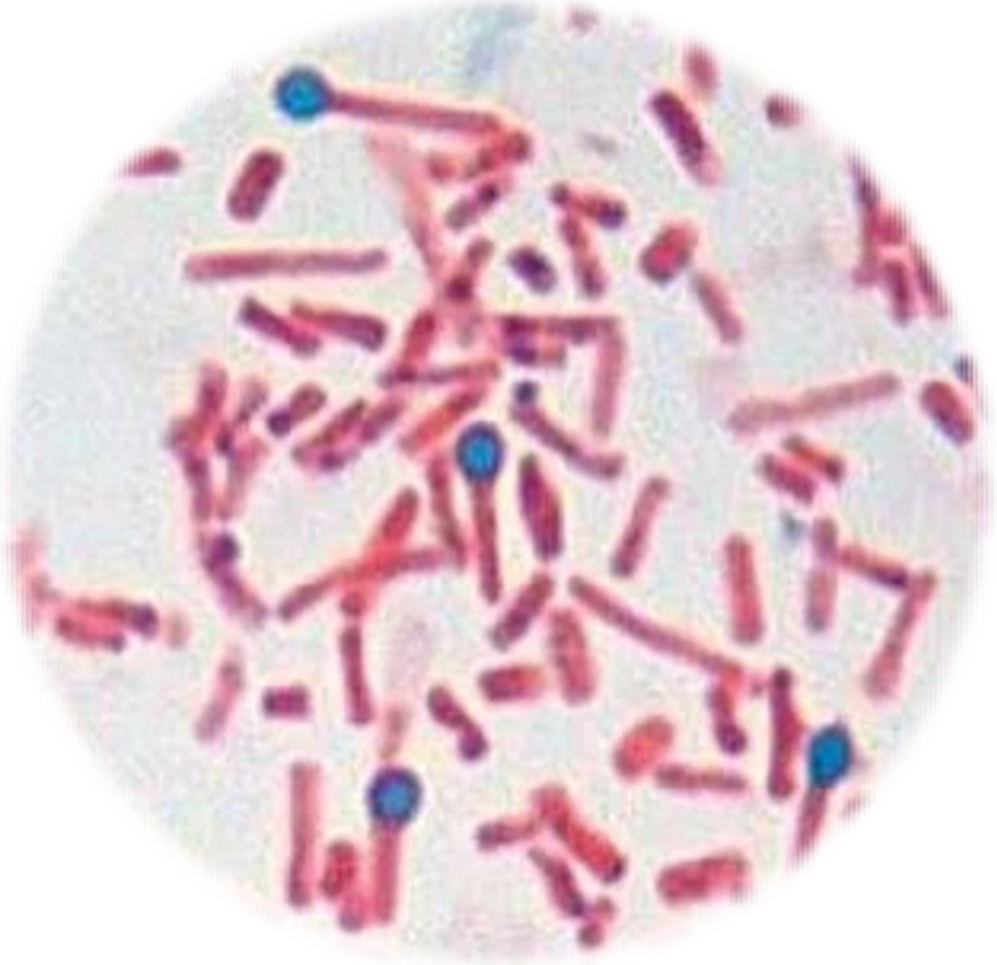


Bacillus anthracis



Clostridium botulinum

Clostridium perfringens



Clostridium tetani

Endospores Positions

The position of the endospore within the cell is a **key feature for bacterial identification**

Terminal

Located at the ends of the bacterial cell.

Clostridium tetani

Subterminal

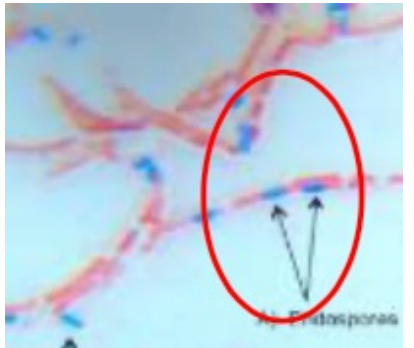
Positioned between the centre and the end of the cell.

Clostridium perfringens

Central

Located in the middle of the bacterial cell.

Bacillus cereus



Endospores staining

- **Challenge:** Visualizing endospores **is difficult** because the endospore wall is impermeable to standard dyes.
- **Method:** The **Schaeffer-Fulton method** is the widely used technique.
- **Dyes: Malachite green** is used as the **primary stain**, and **Safranin** is the **counterstain**.
- **Requirement: Heat must be used** to allow the stain to penetrate the spore.

❖ Staining Procedure

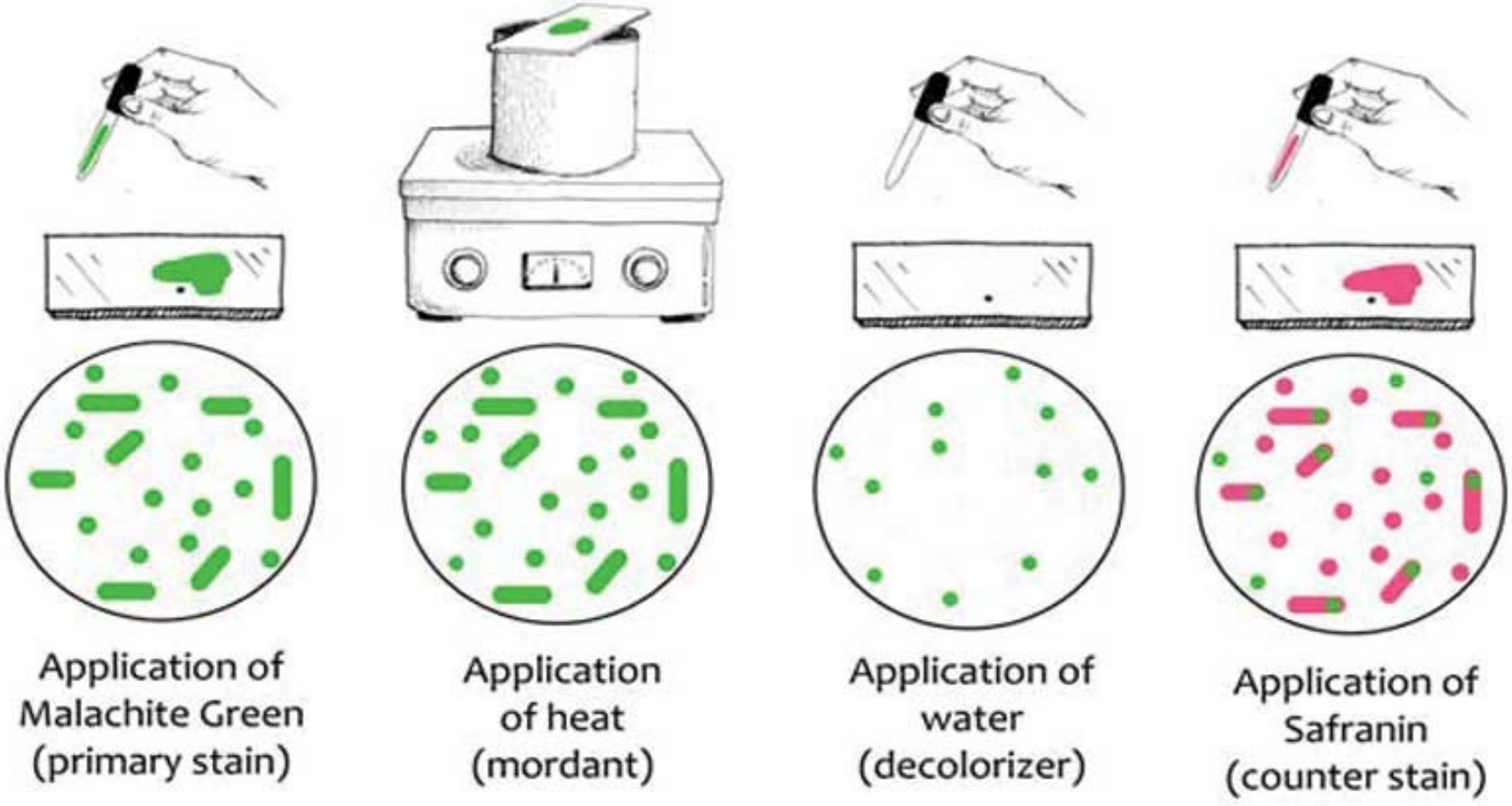
1. **Preparation:** Prepare a **bacterial smear**, air dry, and heat-fix it.

2. **Primary Staining:** Flood the slide with **malachite green** and **steam/heat** for 5–8 minutes to facilitate penetration.

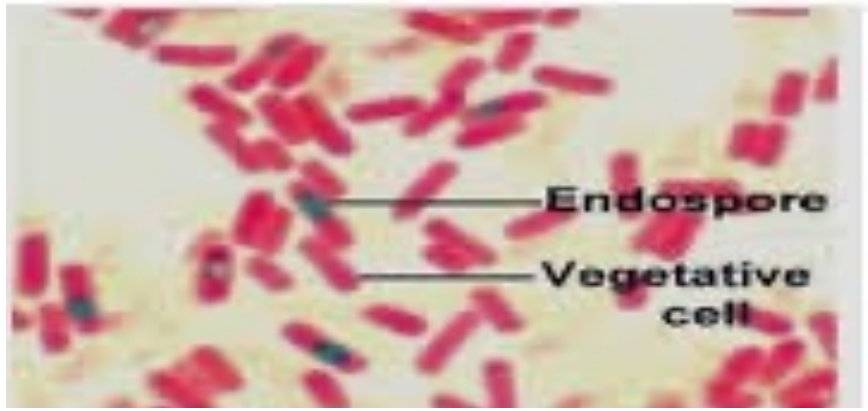
3. **Decolorization:** Rinse the slide with **water** to remove excess malachite green.

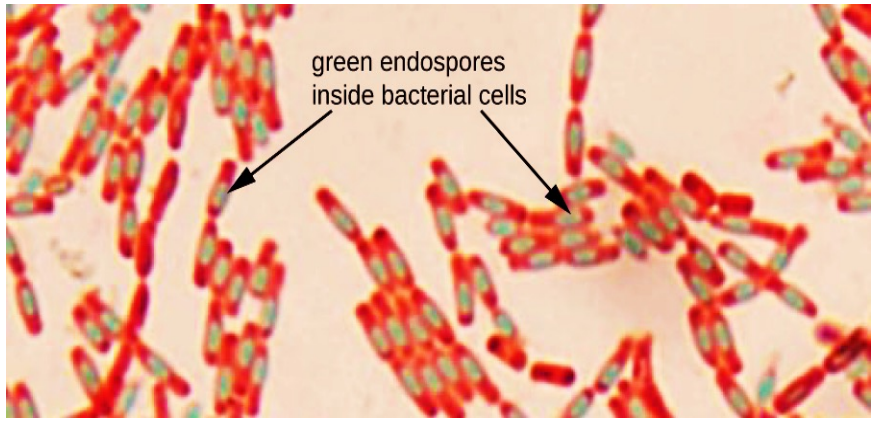
4. **Counterstaining:** Stain with **safranin** for about 2 minutes.

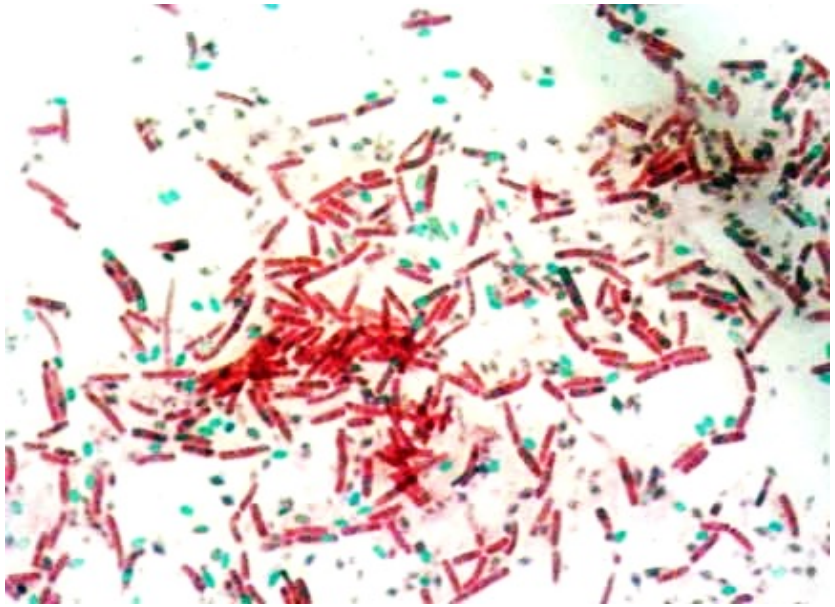
5. **Observation:** Under the microscope, endospores will appear **green**, while vegetative cells will appear **red or pink**.


Application of Malachite Green (primary stain)
Application of heat (mordant)
Application of water (decolorizer)
Application of Safranin (counter stain)

❖ Results


Endospore
Vegetative cell


green endospores
inside bacterial cells



"Success in this course comes from practice, attention to detail, and responsibility in the laboratory. Engage actively and make the most of every practical session.."

End of the Lab 🤓