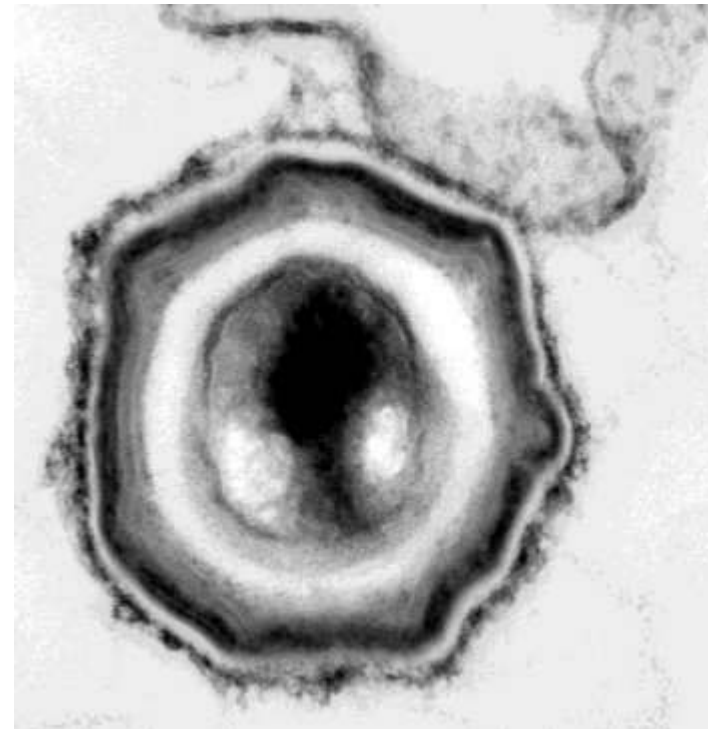
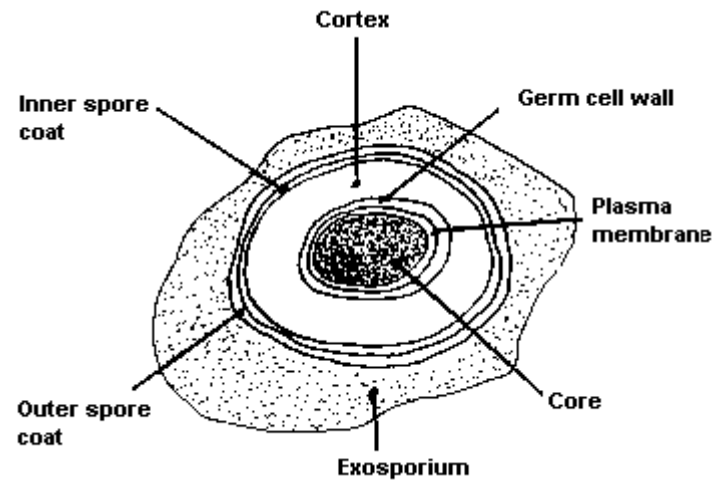
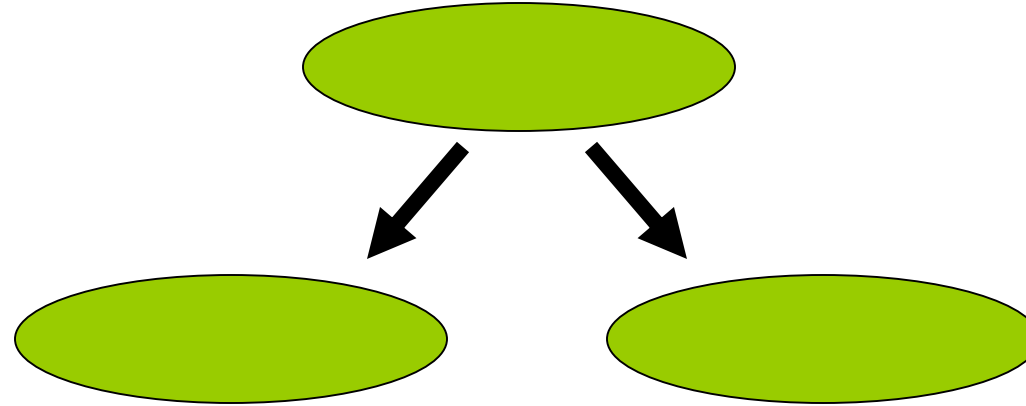


362 MIC  
6<sup>th</sup> Lab  
Bacterial Spores



# Warm-Up



- If a bacteria cell undergoes binary fission, producing an identical copy of itself, what happens to the original parent cell?
  - Does it still exist?
  - Is it one of the daughter cells? Which one?

# Spore Survival

- Most bacteria cannot survive in extreme environmental conditions, such as:
  - Nutrition deprivation
  - Dehydration
  - Temperature extremes
  - Harsh chemicals



# Bacterial Endospores

Endospores: a dormant stage of some bacterium that allows it to survive conditions that would normally kill bacteria such as extreme drought or heat

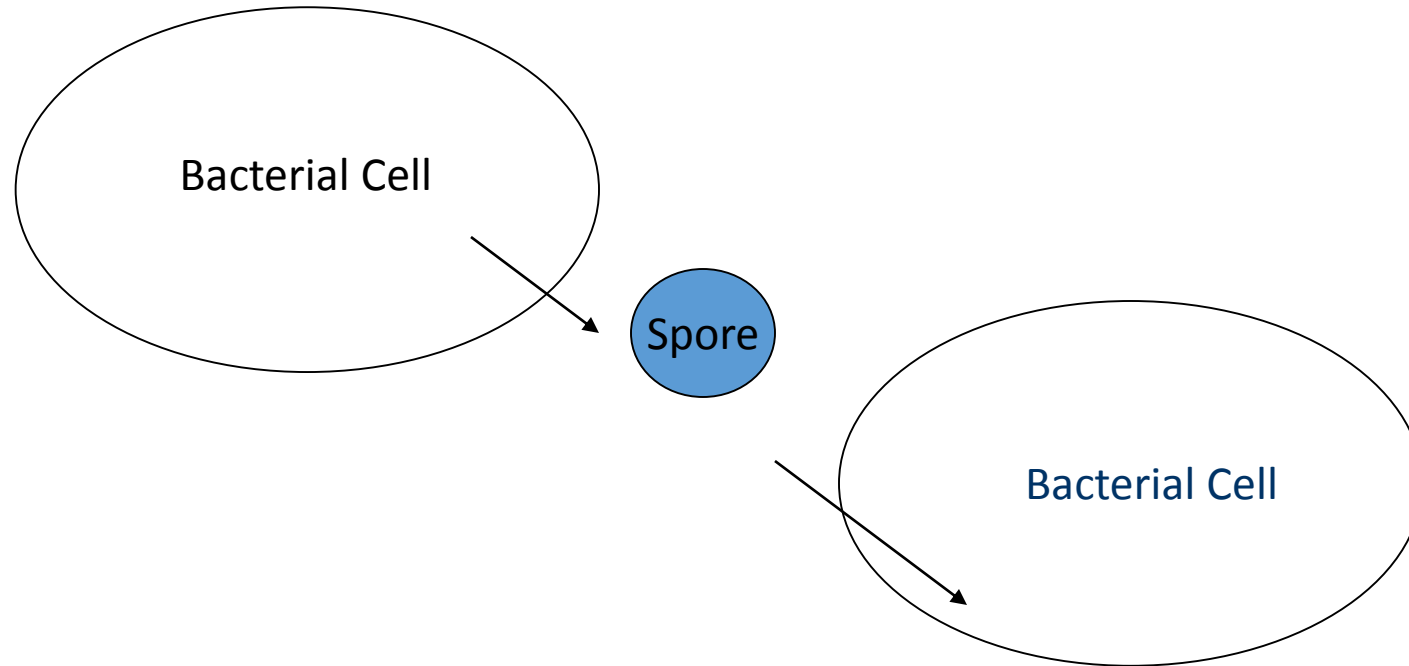
Endospores provide resistance against:

- Drying
- Low nutrient conditions
  - Radiation
  - High temperatures and various chemical disinfectants

Endospores form within the Cell



# The Vegetative Cell Gives Rise to One Spore



The endospore is able to survive for long periods of time until environmental conditions again become favorable for growth. The endospore then germinates, producing a single vegetative bacterium.

# Endospore Function

- Endospores are ultimately protection for the bacterial genome
- Spores form within the cell and contain a full copy of the bacterium's genome
- Endospores are not a form of reproduction, because only one new cell germinates from each spore
- Spores can be variable in size and location within the cell

## **Endospores**

Produced by gram positive bacteria(*Bacillus, Closteridia, Sporosarcinia*)

and *Coxiella burnetii*.

## **Sporulation**

Occurs at the end of the log phase

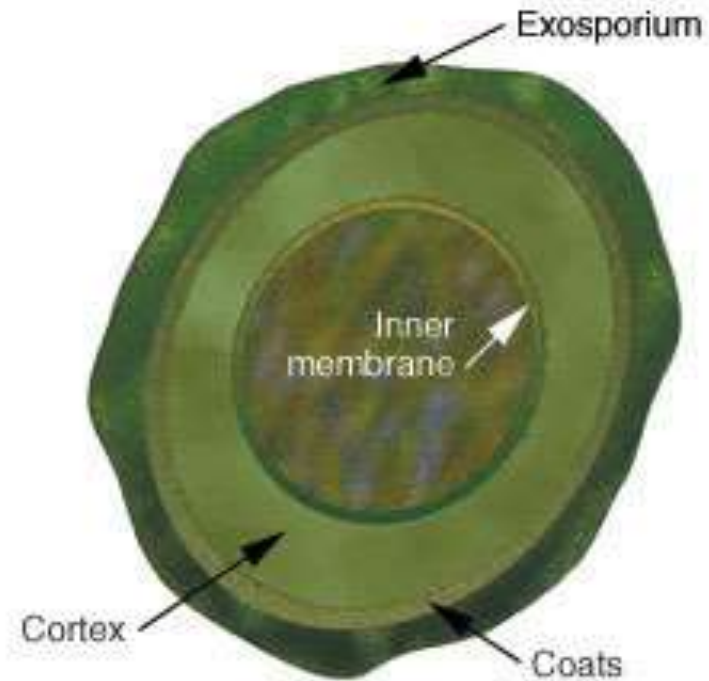
## **Germination**

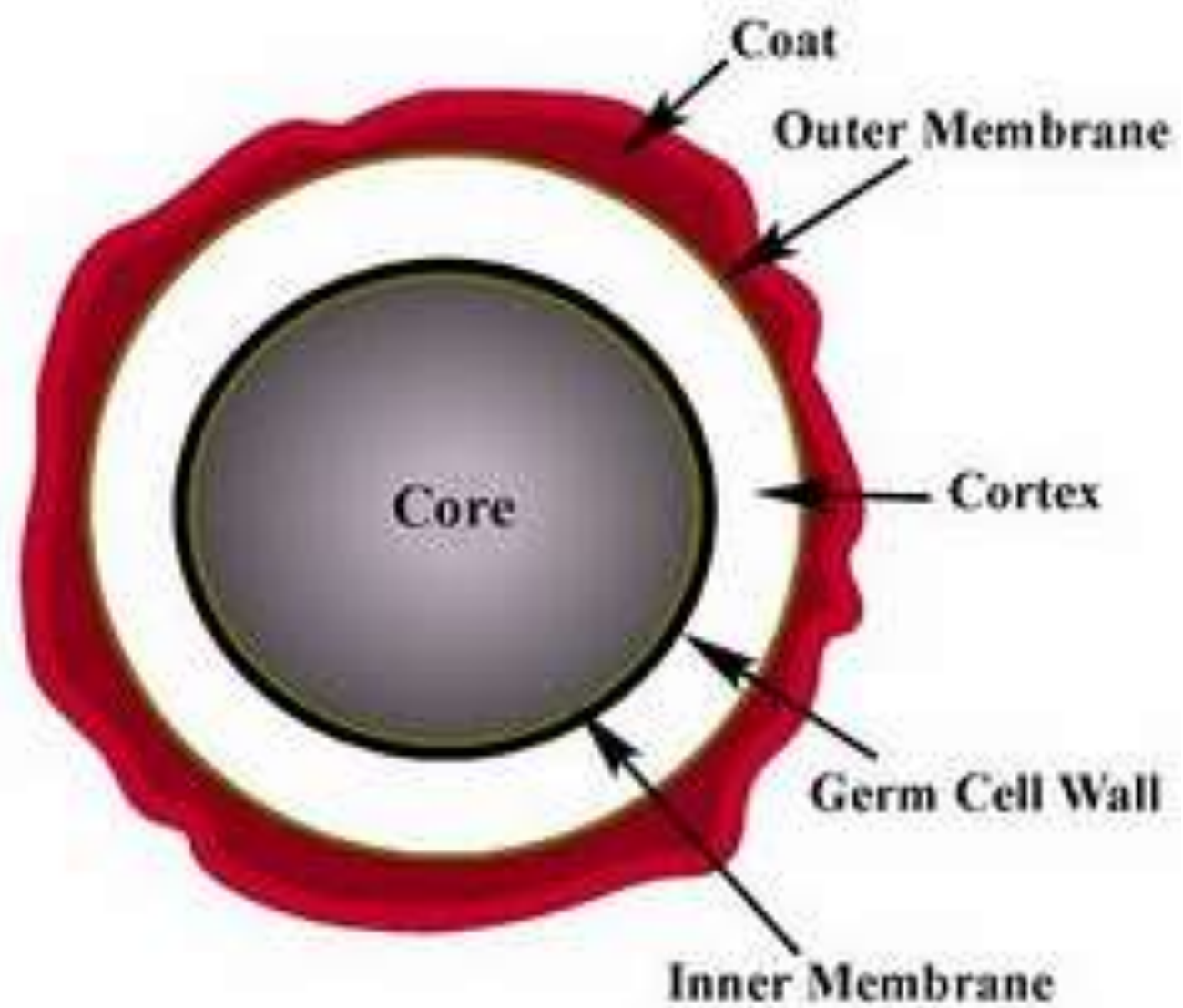
- Activation - Initiation – Outgrowth.



# Spores Structure

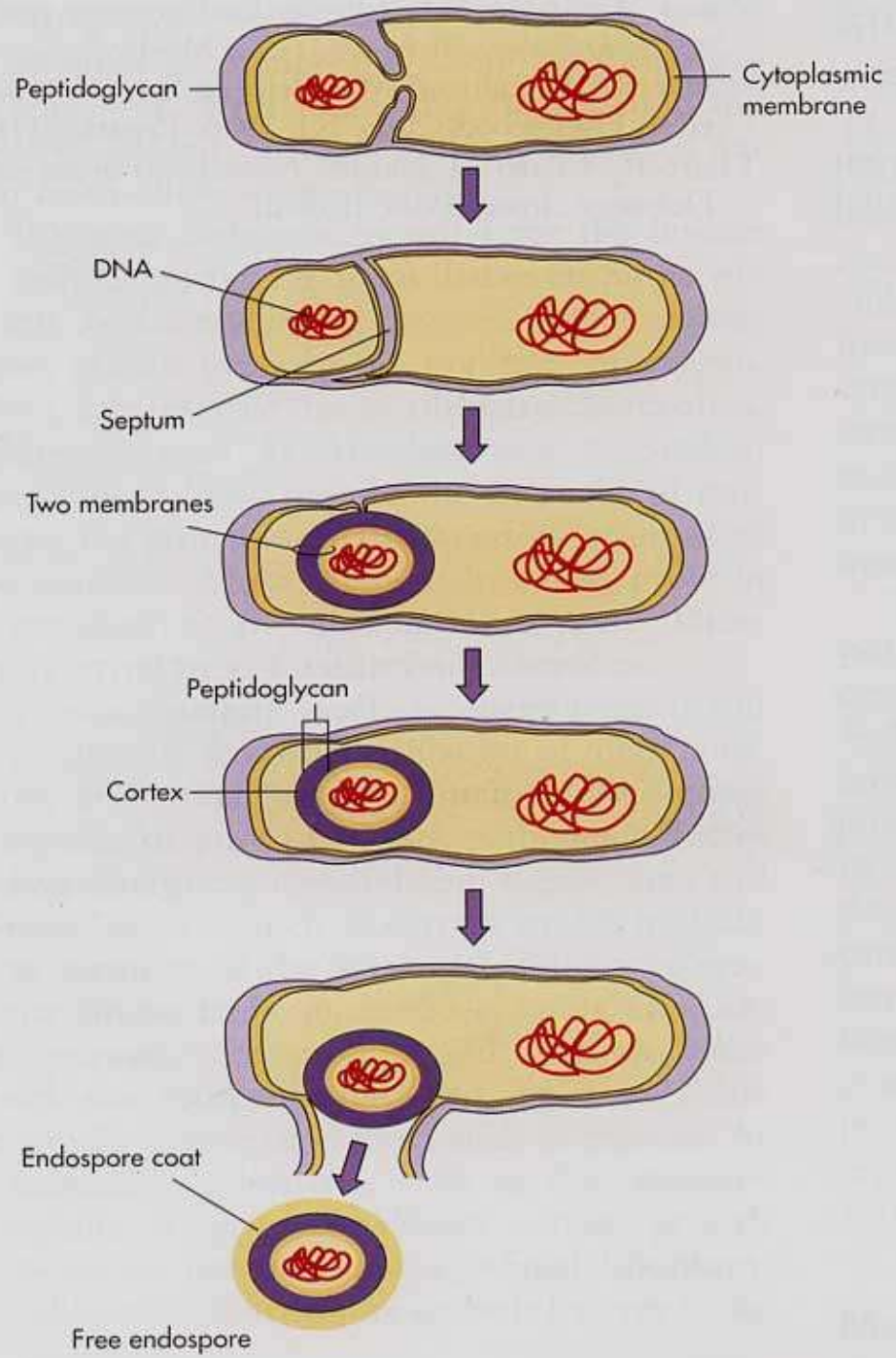
- Spore Core – contains DNA and cytoplasm
- Core Membrane – similar to cell membrane
- Cortex – thickest spore layer, made of loose peptidoglycan
- Inner/Outer Membranes (Spore Coat) – made of protein, impermeable, protects spore from chemicals
- Exosporium Basal Layer – made of glycoprotein and lipoproteins

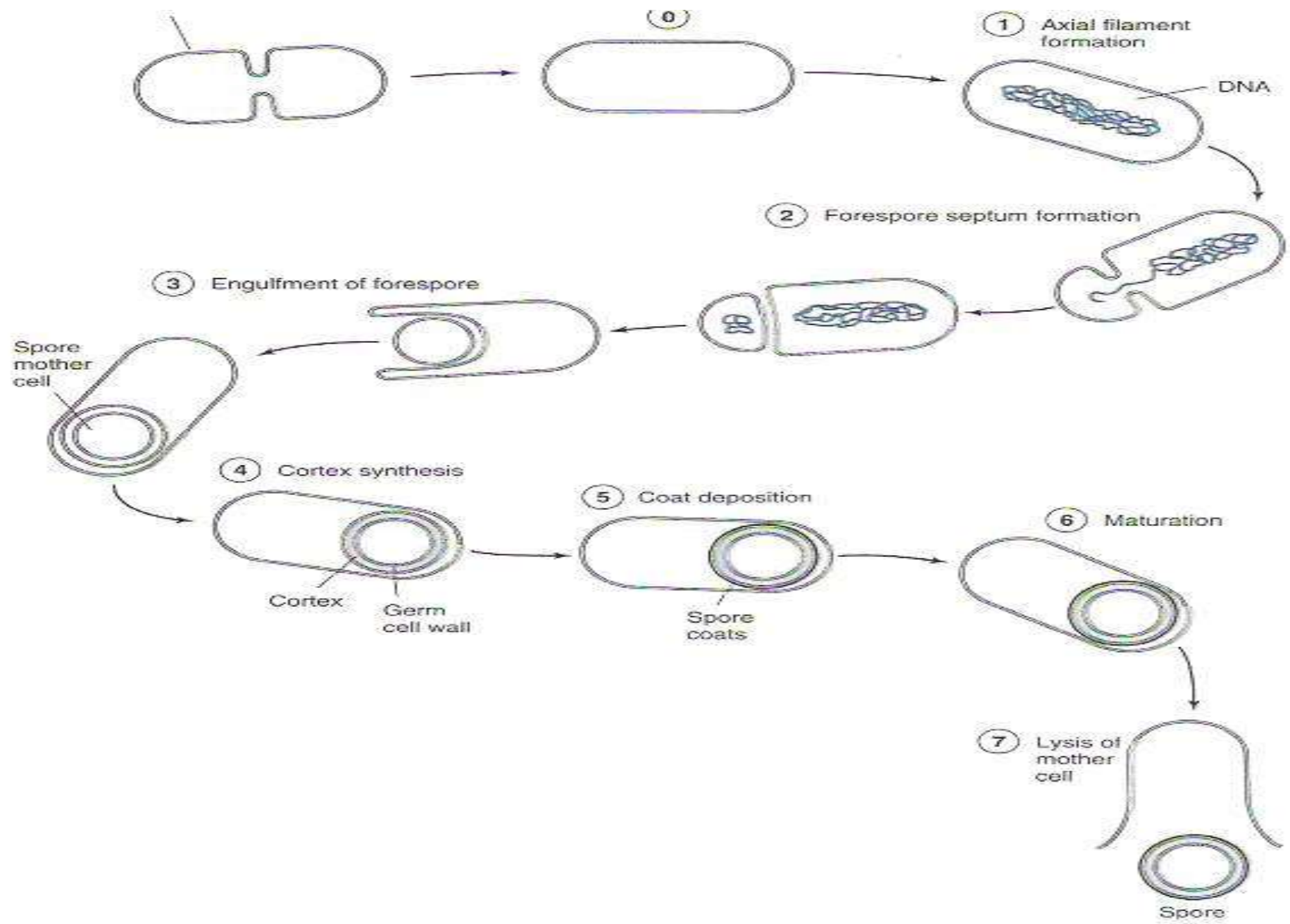




# Sporulation

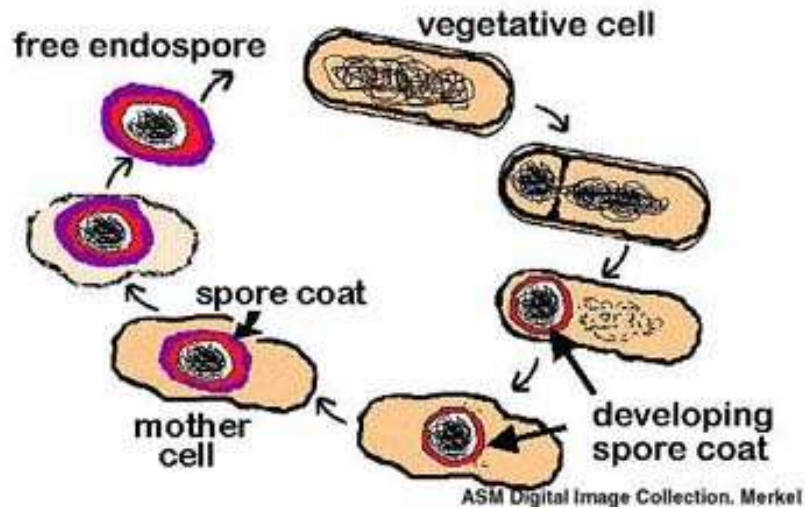
1. DNA replicates and separates, spore septum begins to form
2. Double membrane encloses the DNA and cytoplasm
3. Double membrane matures and becomes core wall
4. Mother cell swells
5. Spore coat develops
6. Exosporium basal layer develops





**Figure 2-32. The stages of endospore formation.** (Reproduced, with permission, from Merrick MJ: *Streptomyces*, in: *Developmental Biology of Prokaryotes*. Parish JH [editor]. Univ California Press, 1979.)

# Germination



- When environmental conditions become favorable again, the dormant spore returns to its vegetative state
- Spore needs to be stimulated (activated) by an environmental stressor before germination begins
- Bacteria cell resumes metabolic activity and spore coat ruptures

# Bold Claims

- Spores recovered from mummies have been brought back to its vegetative state
- Spores found inside bees that were entombed in amber for 25 million years have been reanimated
- Able to survive the  $3000^{\circ}\text{C}$  flame of a rocket and  $-269^{\circ}\text{C}$  of liquid helium



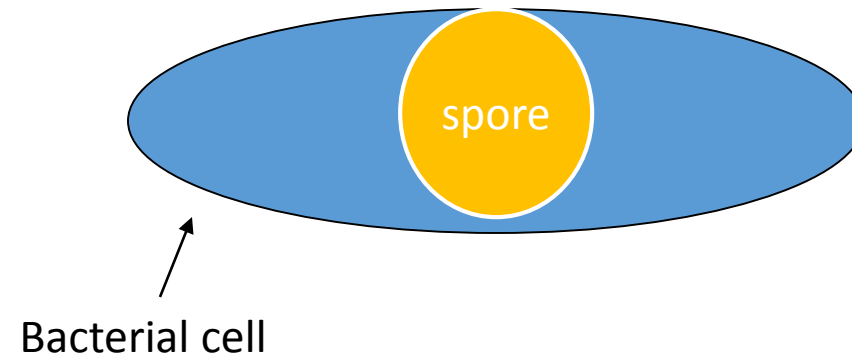
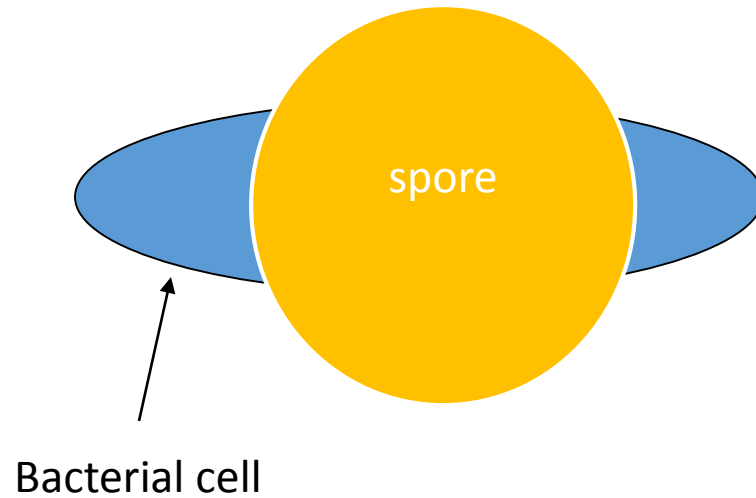
# Not all bacterial species can form spores

- A few genera of bacteria produce Endospore such as Clostridium (gangrene) and Bacillus (anthrax), both of them are gram + rods
- Endospore production is associated with Gram Positive bacteria
- Since not all bacteria form endospores, we can use this as an identification factor



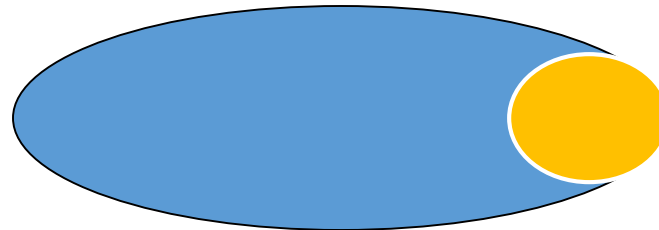
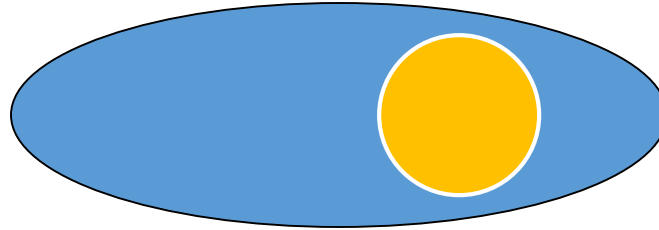
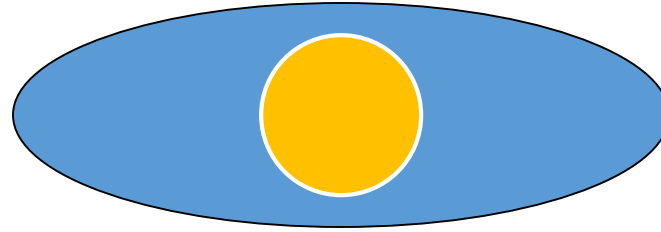
# The shape of the spore is an identifying characteristic

- Swelled vs. Not swelled

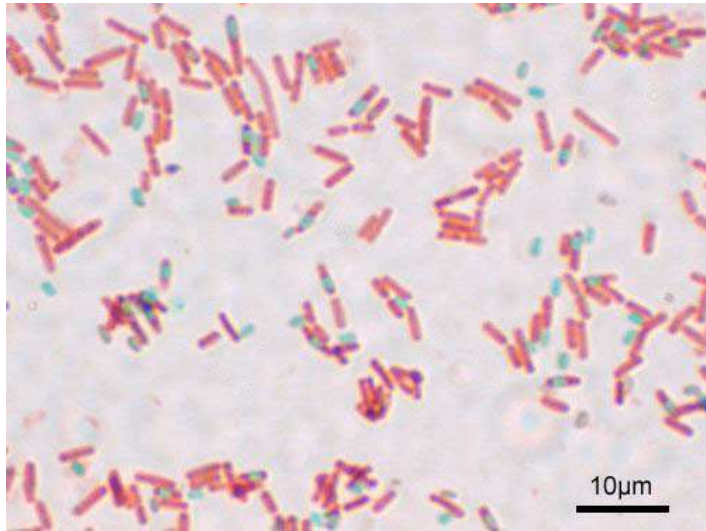


# The location of the spore is also an identifying characteristic

- Central, Sub-Terminal, and Terminal spores



# Bacterial Spores



- Some Gram-positive bacteria can resist extreme conditions by forming an endospore
- Endospores (spores) are tough, dormant structures that allow bacteria to survive environmentally stressful periods
- Spores can lie dormant for thousands of years

## SPORE FORMING BACTERIA

Spores from woodland pond. Image taken using a phase contrast



Some G+ bacteria form resistant structures called SPORES under adverse conditions. Spores are the most **RESISTANT** life form known. They are able to survive boiling in water at 100°C for long periods. Spores are resistant to UV-light, to drying and many harmful chemicals. We know spores can live for 100s of yr. and recently spores several million yr. old have been revived from insects trapped in amber. Some disease organisms like anthrax and botulism form spores that reside in the soil. The size, shape, and location of a spore in the cell are all identifying genetic characteristics.

# Some spore forming bacteria are capable of causing disease

- *Clostridium botulinum* – botulism
- *Clostridium perfringens* – gas gangrene
- *Clostridium tetani* – tetanus
- *Bacillus anthracis* – Woolsorter's Disease and wound infections
- The Schaeffer-Fulton Stain Procedure is used to differentiate between endospores and vegetative cells

# Schaeffer-Fulton Stain Procedure

1. Make a smear. Air Dry. Heat fix
2. Flood the smear with Malachite Green stain
3. Cover the flooded smear with a square of filter paper
4. Steam slide for 10 minutes (every minute, add a few more drops of Malachite Green stain)
5. Allow slide to cool (after the 10 min. steam process)

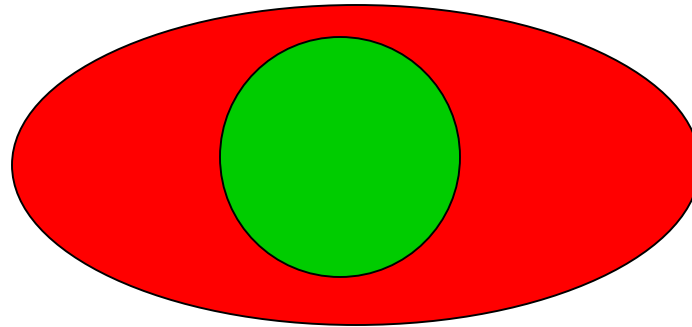
## Schaeffer-Fulton Stain Procedure (continued)

6. Drain slide and rinse for 30 seconds with DI water (discard filter paper)
7. Put slide on steam rack
8. Flood smear with Safranin (counter stain). This stains the vegetative cell. (Leave for 1 minute)
9. Drain the slide and rinse with DI water
10. Blot Dry
11. Use oil immersion objective to view

# Endospore Stain Example

Spores: Green

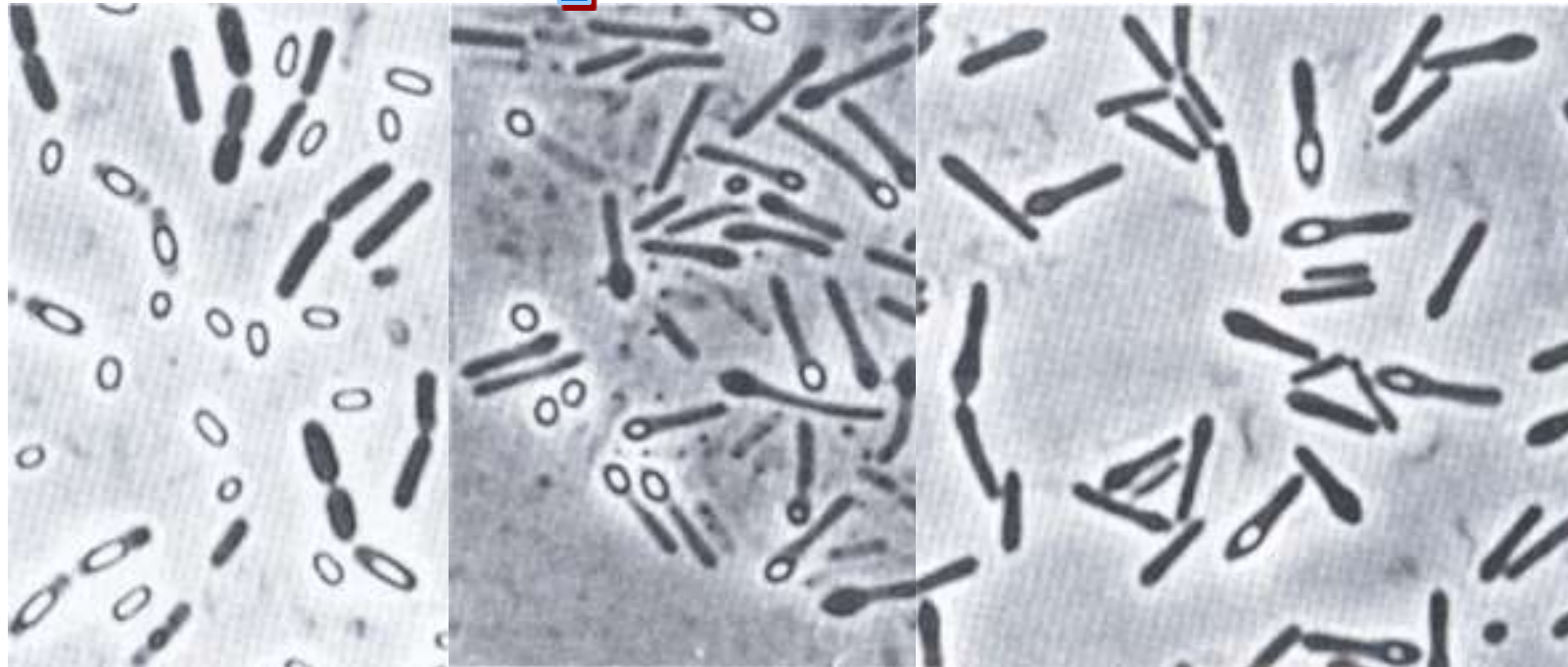
Cell: Red or Pink



Each Person will make a smear and Endospore stain  
of: *Bacillus pumilus* or *Bacillus subtilis*

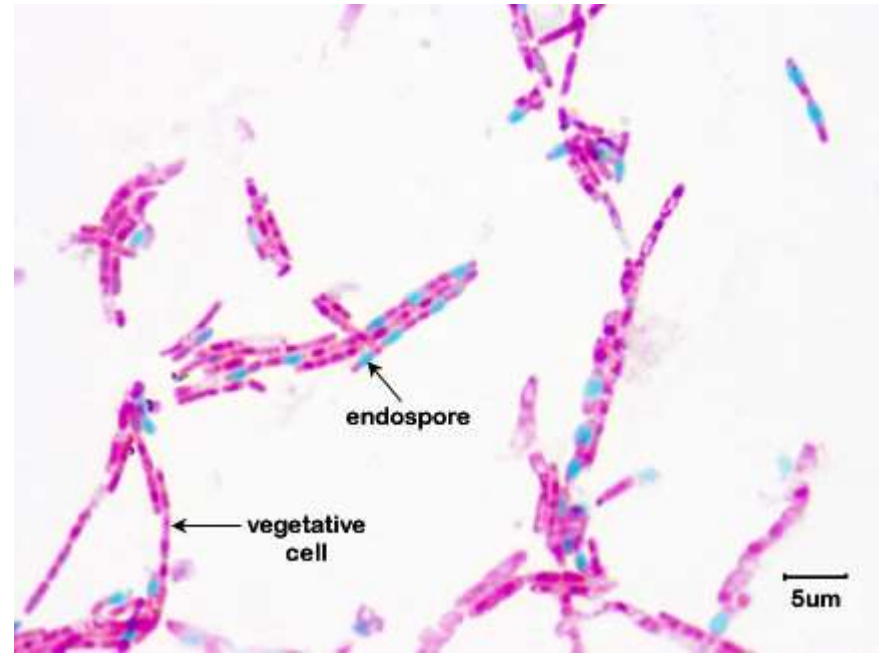
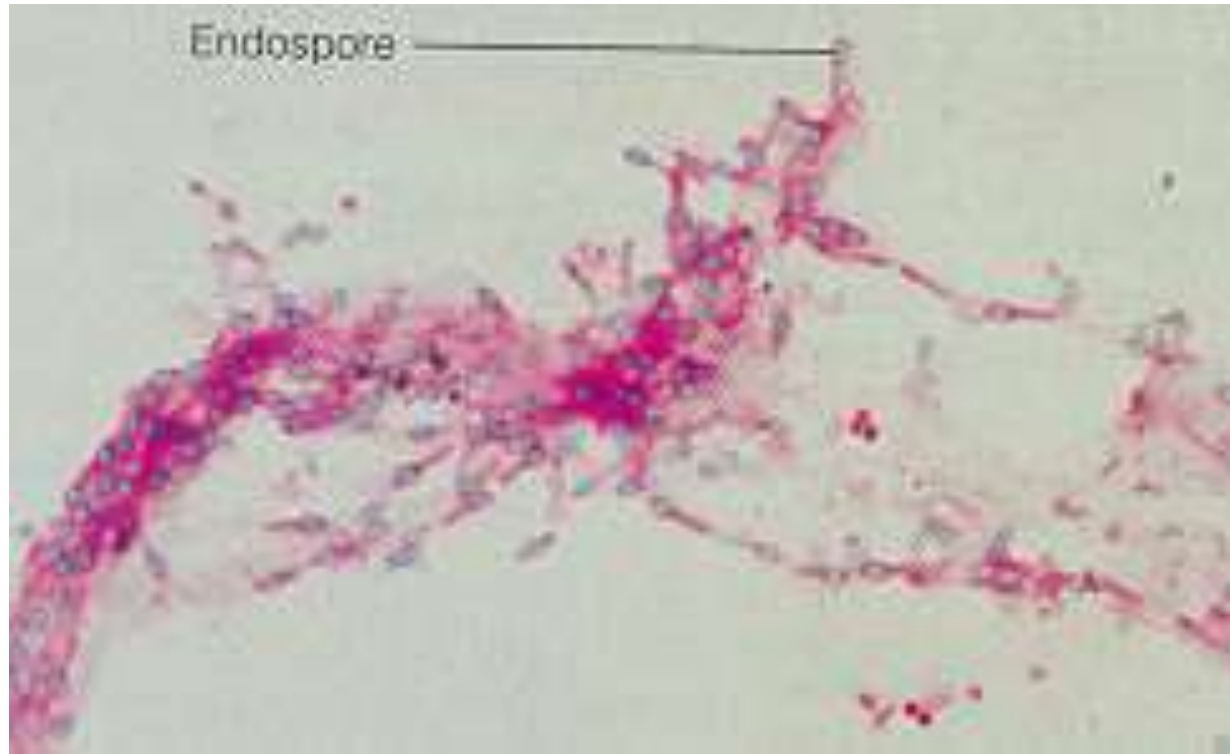


# Endospore Pictures



Centrally (left), terminally (center), and subterminally (right) located endospores.

Pictures from Brock Biology of Microorganisms (lecture textbook)



# What do you think?

- If bacterial spores are able to resist such harsh conditions, how do we destroy them?