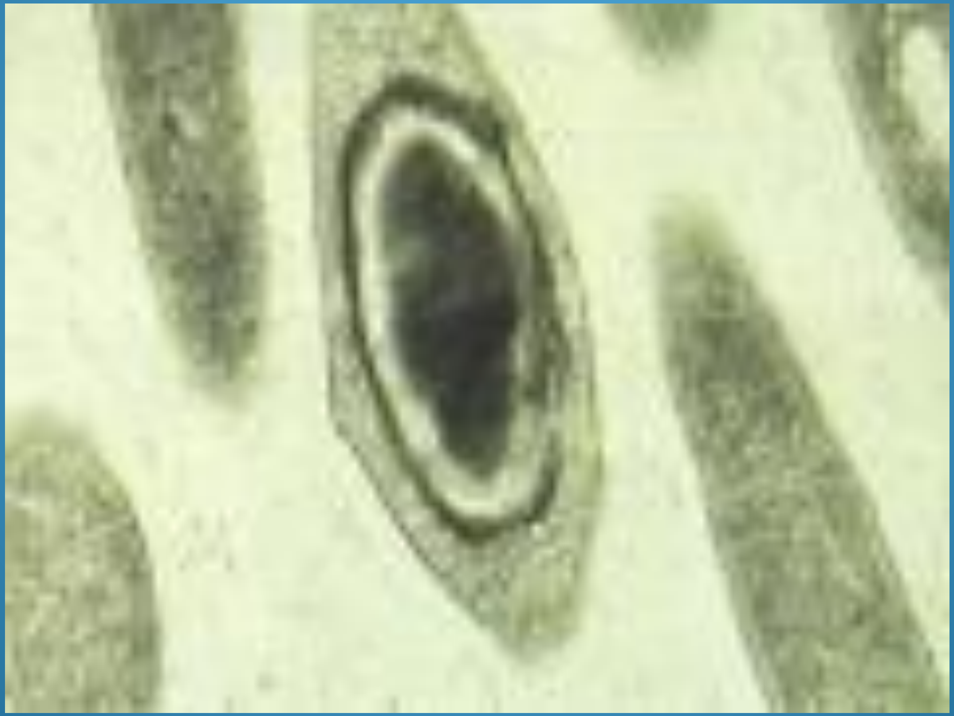
Bacterial Spores (endospore)



Endospores form within the Cell

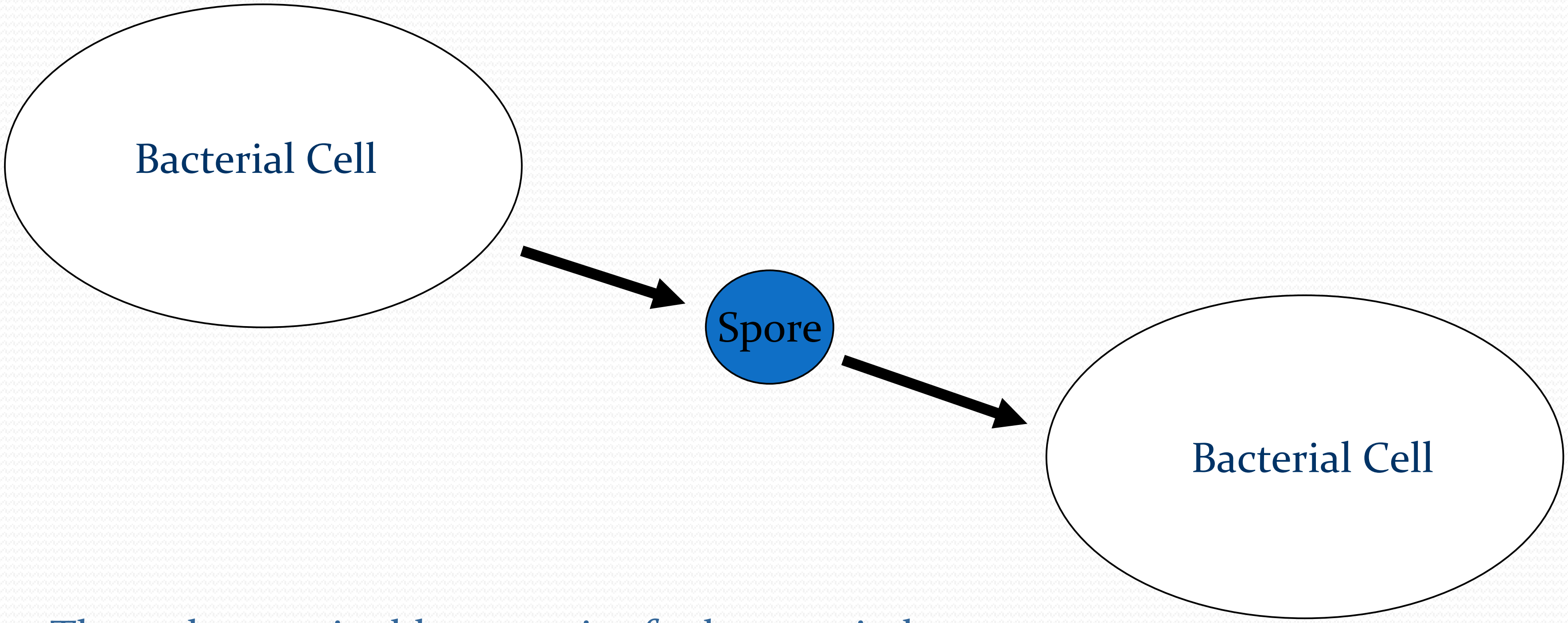
- Endospore is dormant stage of some bacterium that allows it to survive unfavorable conditions that would normally be lethal such as extreme drought or heat
- Endospores are resistant against;
  - Drought
  - Low nutrient conditions
  - Radiation
  - High temperatures
  - Various chemical disinfectants

Endospores

- The spore is a dehydrated, multishelled structure that protects and allows the bacteria to exist in “suspended animation”

- It contains a complete copy of the chromosome, the bare minimum concentrations of essential proteins and ribosomes, and a high concentration of calcium bound to dipicolinic acid.

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.

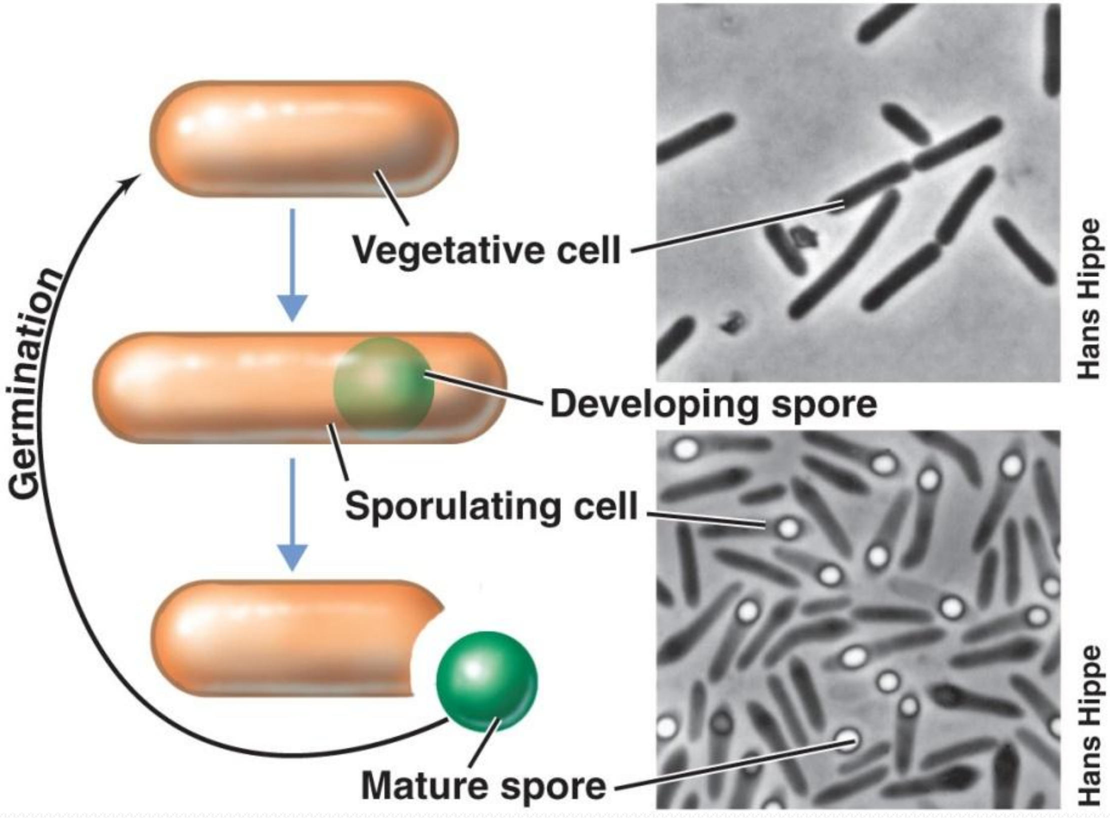
The endospore then germinates, producing a single vegetative bacterium.

Endospore Function

- Endospores are ultimately protection for the bacterial genome
- Spores form within the cell and contain a full copy of the bacterial 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

Sporulation or Sporogenesis

- Process of endospore formation within a vegetative (parent) cell
- Germination = return of an endospore to its vegetative state



Sporulation

- The sporulation process begins when nutritional conditions become unfavorable, depletion of the nitrogen or carbon source (or both) being the most significant factor.

- Sporulation occurs massively in cultures that have terminated exponential growth as a result of such depletion.

- Sporulation involves the production of many new structures, enzymes, and metabolites along with the disappearance of many vegetative cell components.
    - These changes represent a true process of differentiation. A set of genes whose products determine the formation and final composition of the spore are activated, while another subset of genes involved in vegetative cell function are inactivated.
    - These changes involve alterations in the transcriptional specifity of RNA polymerase, which is determined by the association of the polymerase core protein with one or another promoter-specific protein called a sigma factor. Different sigma factors are produced during vegetative growth and sporulation.

Sporulation

- Morphologically, sporulation begins with the isolation of a terminal nucleus by the inward growth of the cell membrane.

- The growth process involves an infolding of the membrane so as to produce a double membrane structure whose facing surfaces correspond to the cell wall-synthesizing surface of the cell envelope. The growing points move progressively toward the pole of the cell so as to engulf the developing spore.

Sporulation

- The two spore membranes now engage in the activity synthesis of special layer that will form the cell envelope:
  - the spore wall and cortex, lying between the facing membranes, and the coat and exosporium lying outside the facing membrane.
- In the newly isolated cytoplasm, or core, many vegetative cell enzymes are degraded and are replaced by a set of unique spore constituents.

http://www.youtube.com/watch?v=i2m2-_YkN5Y

Table 4.3 Differences between endospores and vegetative cells

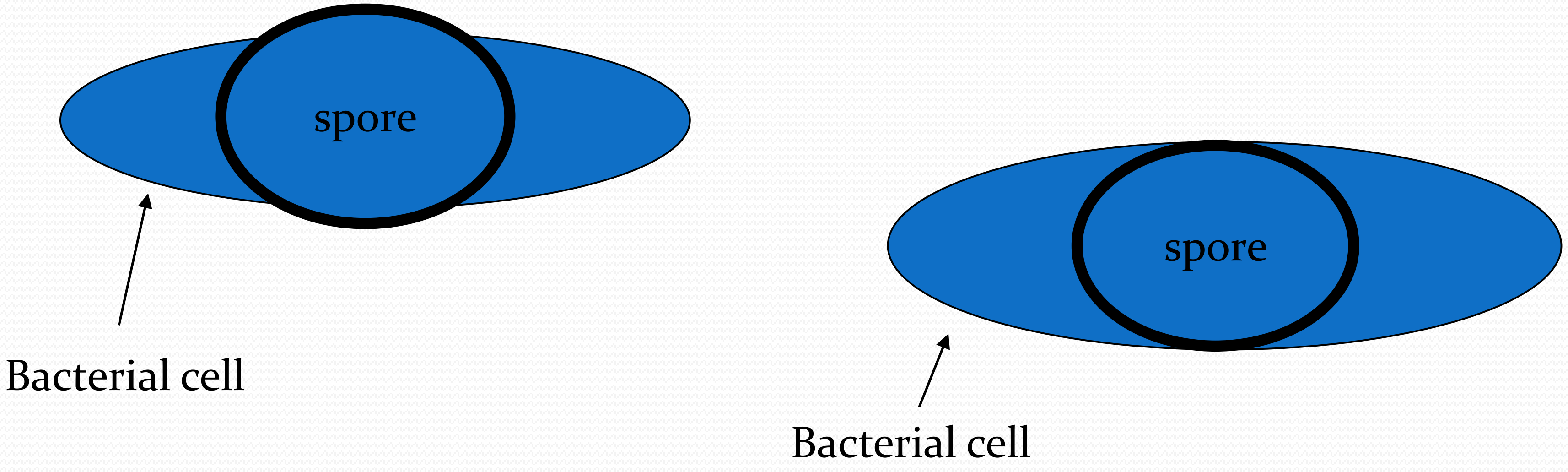
Characteristic	*Vegetative cell*	*Endospore*
Structure	Typical gram-positive cell; a few gram-negative cells	Thick spore cortex; Spore coat; exosporium
Microscopic appearance	Nonrefractile	Refractile
Calcium content	Low	High
Dipicolinic acid	Absent	Present
Enzymatic activity	High	Low
Metabolism (O_2 uptake)	High	Low or absent
Macromolecular synthesis	Present	Absent
mRNA	Present	Low or absent
DNA and ribosomes	Present	Present
Heat resistance	Low	High
Radiations resistance	Low	High
Resistance to chemicals (for example, H_2O_2) and acids	Low	High
Stainability by dyes	Stainable	Stainable only with special methods
Action of lysozyme	Sensitive	Resistant
Water content	High, 80–90%	Low, 10–25% in core
Small acid-soluble proteins (product of *ssp* genes)	Absent	Present
Cytoplasmic pH	About pH 7	About pH 5.5–6.0 (in core)

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

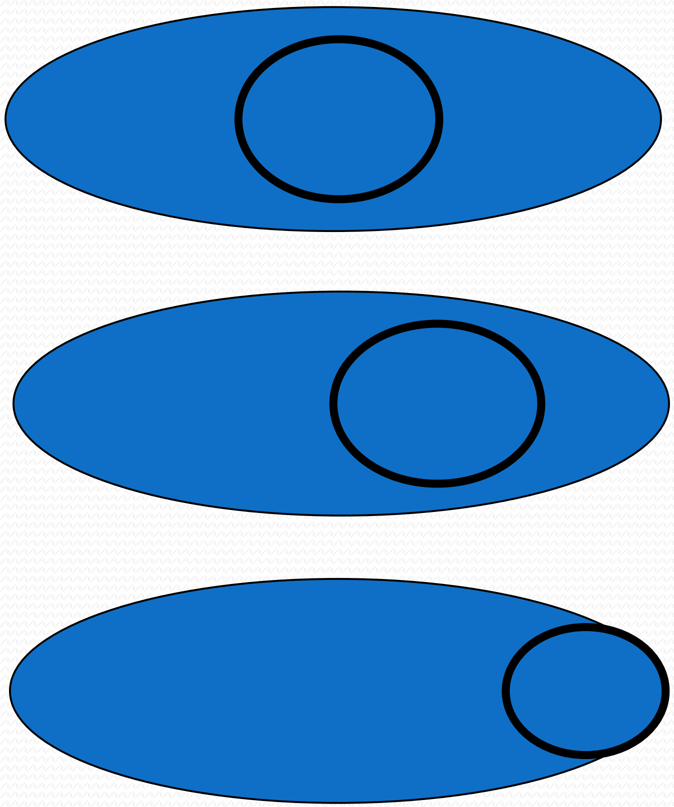
The shape of the spore is an identifying characteristic

- Swelled vs. Not swelled



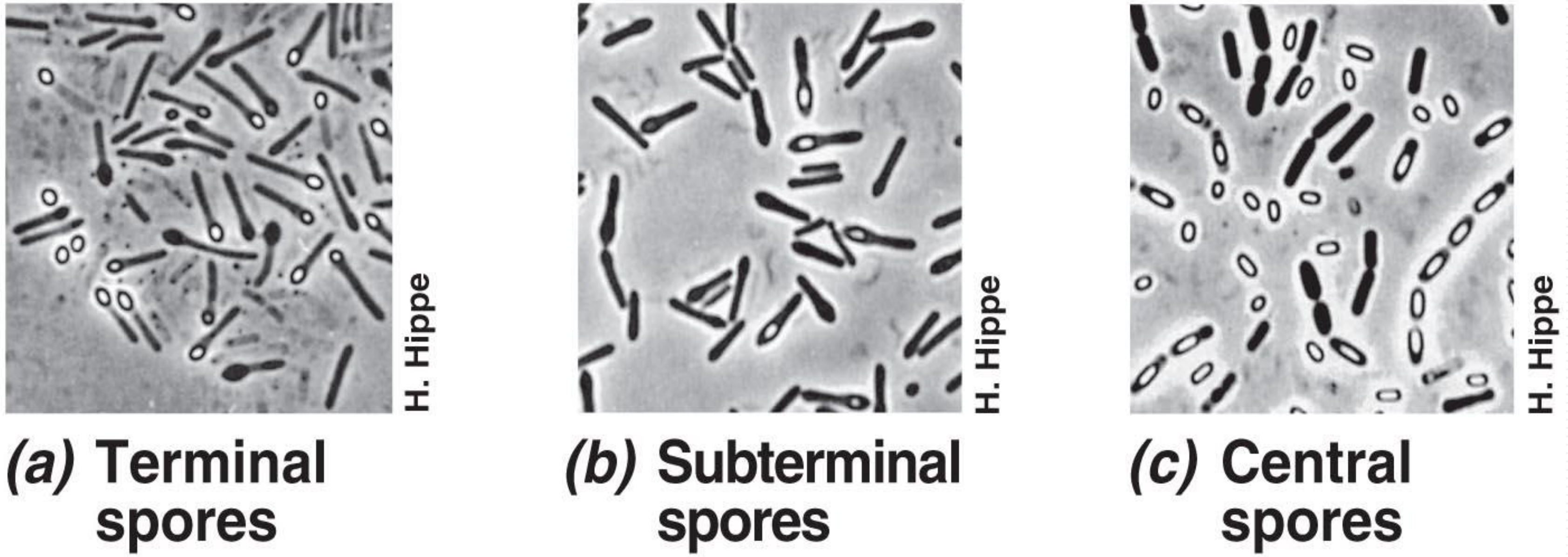
The location of the spore is also an identifying characteristic

- Central, Sub-Terminal, and Terminal spores



Endospores

- Endospores can remain dormant indefinitely but germinate quickly when the appropriate trigger is applied
- Endospores differ significantly from the vegetative, or normally functioning, cells



Some spore forming bacteria are capable of causing disease

- *Clostridium botulinum* – botulism
- *Clostridium perfingens* – 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)

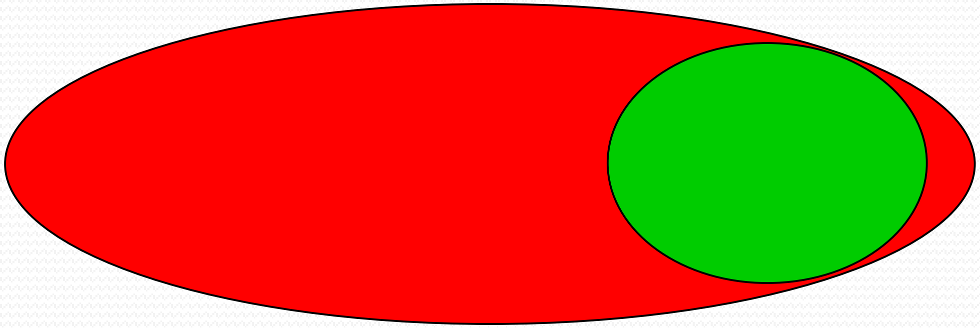
Schaeffer-Fulton Stain Procedure (cont'd)

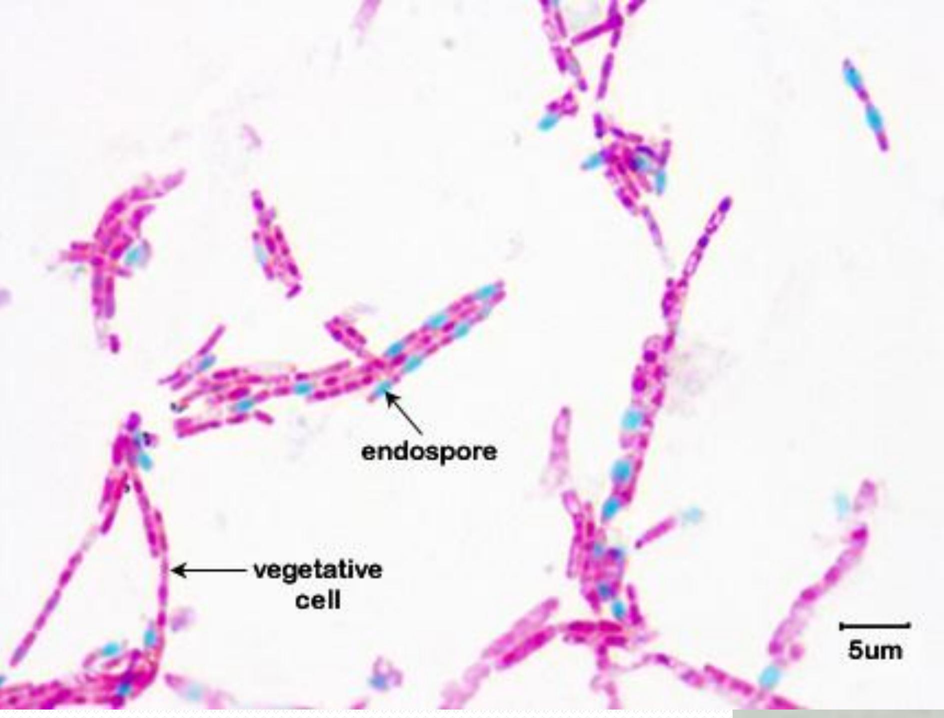
6. Drain slide and rinse for 30 seconds with DI water (discard filter paper)
7. Put slide on steam rack
8. Flood smear with Safranin (counter stain). This stains the vegetative cell. (Leave for 1 minute)
9. Drain the slide and rinse with DI water
10. Blot Dry
11. Use oil immersion objective to view

Endospore Stain Example

Spores: Green

Cell: Red or Pink




endospore
vegetative cell
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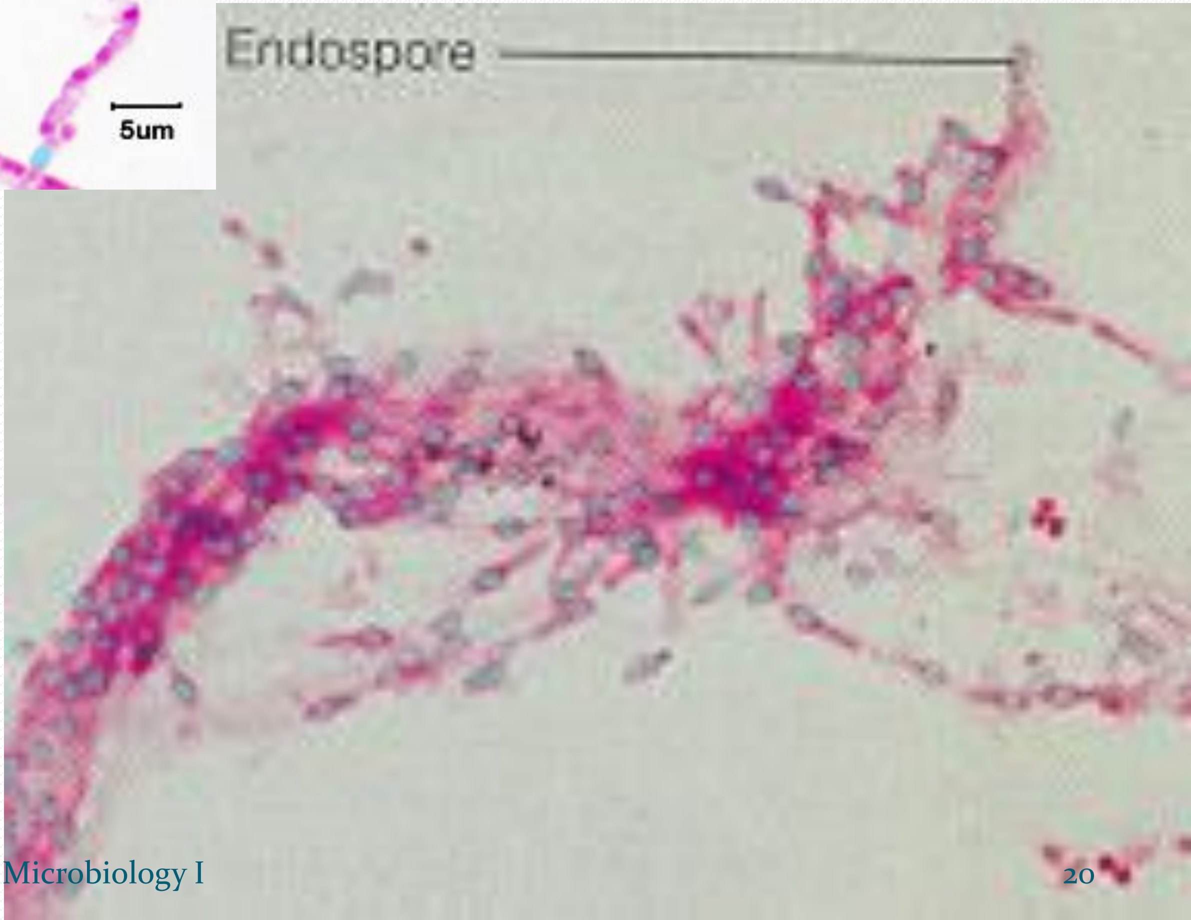
Endospore

Spore Germination

- Activation by heat and nutrients
- Ca-dipicolinate and cortex components disappear
- SASPs degrade
- Swelling with H_2O
- Cell begins to divide like normal
- *Bacillus anthracis* (and *Clostridium*) produces endospores
  - Easily aerosolized and spread
  - Relatively easy and inexpensive to prepare in laboratory
  - Can be easily transported without detection

Tyndallization

- by John Tyndall (1820-1893)

- Boil for 15 min
- Keep in warm, humid environment for 1 d
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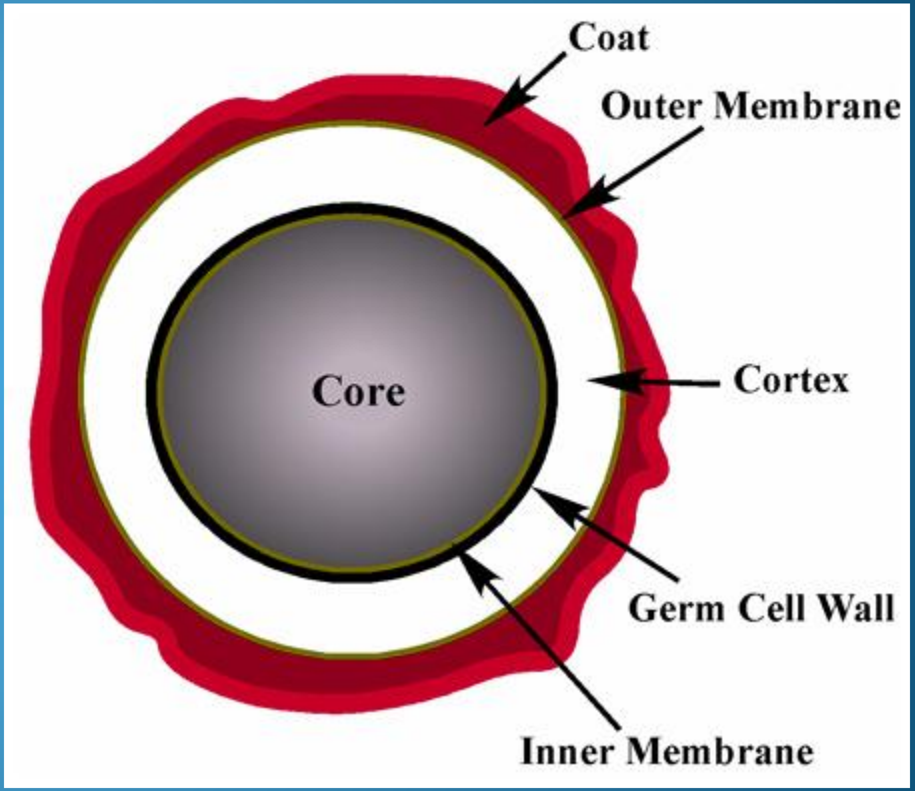
Properties of endospores



Core

- The core is the spore protoplast.
- It contains a complete nucleus (chromosome), all of the components of the proteins-synthetizing apparatus, and an energy-generating system based on glycolysis. Cytochromes are lacking even in aerobic species, the spores of which rely on shorted electron transport pathway involving flavoproteins. A number of vegetative cell enzymes are increased in amount (eg. alanine racemase), and a number of unique enzymes are formed (eg. dipicolinic acid synthetase).
- The energy for germination is stored as 3-phosphoglycerate rather than as ATP.

Core

- The heat resistance of spores is due in part to their dehydrated state and in part to the presence in the core of large amounts (5 – 15% of the spore dry weight) of calcium dipicolinate, which is formed from an intermediate of the the lysine biosynthetic pathway.

- In some way not yet understood, these properties result in the stabilization of the spore enzymes, most of which exhibit normal heat lability when isolated soluble form.

Spore wall

- The innermost layer surrounding the inner spore membrane is called the spore wall.

- It contains normal peptidoglycan and becomes the cell wall of the germinating vegetative cell.

Cortex

- The cortex is the thickest layer of the spore envelope.
- It contains an unusual type of peptidoglycan, with many fewer cross-links than are found in cell wall peptidoglycan.
- Cortex peptidoglycan is extremely sensitive to lysozyme, and its autolysis plays a key role in spore germination.

Coat

- The coat is composed of a keratin-like protein containing many intramolecular disulfide bonds.
- The impermeability of this layer confers on spores their relative resistance to antibacterial chemical agents.

Exosporium

- The exosporium is a lipoprotein membrane containing some carbohydrate.

Germination

- The germination process occurs in three stages:
  - activation,
  - initiation,
  - outgrowth.

Activation

- Even when placed in an environment that favors germination (eg. nutritionally rich medium) bacterial spores will not germinate unless first activated by one or another agent that damages the spore coat.

- Among the agents that can overcome spore dormancy are heat, abrasion, acidity, and componds containing free sulfhydryl groups.

Initiation

- Once activated, a spore will initiate germination if the environmental conditions are favorable.
- Different species have evolved receptors recognise different effectors as signaling a rich medium.
- Binding of the effector activates an autolysin that rapidly degrades the cortex peptidoglycan. Water is taken up, calcium dipicolinate is released, and a variety of spore constituents are degraded by hydrolytic enzymes.

Outgrowth

- Degradation of the cortex and outer layers results in the emergence of a new vegetative cell consisting of the spore protoplast with its surrounding wall.

- A period of active biosynthesis follows. This period, which terminates in cell division, is called outgrowth.

- Outgrowh requires a supply of all nutrients essenial for cell growth.

The spore stain

- Spores are most simply observed as intracellular refractile bodies in unstained cell suspensions or as colorless areas in cell stained by conventional methods.

- The spore wall is relatively impermeable, but dyes can be made to penetrate it by haeting the preparation.

- The same impermeability then serves to prevent decolorization of the spore by a period of alcohol treatment sufficient to decolorize vegetative cells. The latter can finally be counterstained. Spores are commonly stained with malachite green or carbolfuchsin.