BACTERIAL ENDOSPORES

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STRUCTURAL MODIFICATIONS OF BACTERIAL CELLS

Some bacterial genera have evolved structural modifications as dormant structures under adverse conditions of extreme heat, desiccation, radiation, chemicals, pH etc.

These include:

1.Endospores as in *Bacilli* and *Clostridia*.

2.Exospores (conidiospores) as in *Actinomycetes*.

3.Cysts as in *Azotobacter*.

4.Non-cultivable (but viable) forms as in *Enterobacteriaceae*.

Endospores

- Small, metabolically-inert cells with thick cell walls formed by some Gram positive cells:
  - form in response to adverse environmental conditions
  - resistant to chemical inactivation (NB in sterilisation and infection control)
  - appear as oval or round cells within the vegetative cells

- **Endospores are defined as round or ovoid, thick-walled, highly refractile, metabolically inert resistant structures**
- **Spores are not obligatory part of the bacterial life cycle.**
- **Endospore formation is a mechanism of survival rather than a mechanism of reproduction**
- **Endospores exhibit no signs of life, being described as cryptobiotic.**
- **Although cryptobiotic, they retain viability indefinitely such that under appropriate environmental conditions, they germinate back into vegetative cells.**
- **The endospores can survive possibly hundreds of years until a variety of environmental stimuli trigger germination; they are probably the most durable cells produced in nature.**

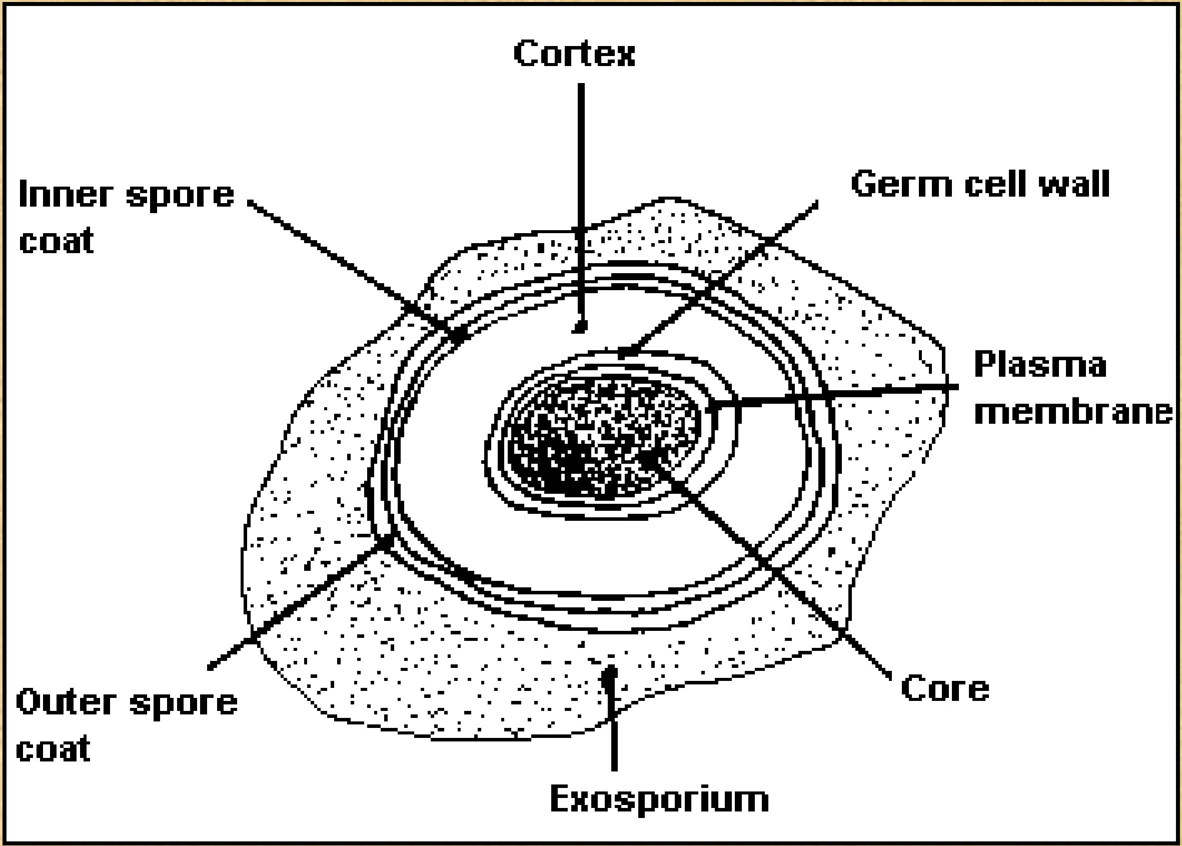
- **Spore formation is usually triggered by detrimental environmental growth conditions, specially limitation of C-supply.**

- **Sporulation occurs in the late log phase / early stationary phase of bacterial growth cycle.**

- **The endospores are produced at the rate of one per cell inside bacteria.**

- **All endospores producing bacteria are Gram-positive bacilli (except *Sporosarcina*) such as *Bacillus, Clostridium, Thermoactinomycetes* and *Sporolactobacillus*.**

STRUCTURE OF ENDOSPORES



The core:

- **innermost area of the spore is the spore protoplast or spore core**
- **consists of a single copy of chromosome, ribosomes, tRNAs, proteins and enzymes**
- **proteins act as a source of amino acids during early phase of germination; they also protect DNA from UV-induced damage.**
- **enzymes serve to start protein synthesis and metabolism during germination**
- **energy is stored in the form of 3-P-glycerate.**
- **contains a unique chemical dipicolinic acid (DPA, pyridine 2-6 dicarboxylic acid), which is absent in vegetative cells. It chelates calcium ions and is responsible for heat resistance of spores.**

Germ cell membrane:

- **the layer immediately surrounding the core (plasma membrane)**

Germ cell wall:

- **made of peptidoglycan**
- **provides osmotic stability to the spore protoplast.**
- **initiates vegetative cell wall formation**

Spore cortex:

- **thick concentric structure outside the cell wall**
- **made of modified peptidoglycan:**
    - **every third muramic acid residue in the glycan chainis replaced by muramic anhydride,**
    - **incomplete peptide chains consisting of only L-alanine, and**
    - **a very low degree of cross linking**
- **cortex appears to play a key role in an energy dependent dehydration of spore during sporulation.**

Spore coat:

- a keratin like thick protein layer situated outside the cortex
- contains approximately 80% of the total spore proteins rich in disulfide groups
- confer resistance against toxic chemicals including antibiotics.

Exosporium:

- an additional loose layer surrounding the cortex in some bacteria
- consists of lipids and glycoproteins.

Differences between endospores and vegetative cells

Property	Vegetative cells	Endospores
Surface coats	Typical Gram-positive murein cell wall polymer	Thick spore coat, cortex, and peptidoglycan core wall
Microscopic appearance	Non-refractile	Refractile
Calcium dipicolinic acid	Absent	Present in core
Cytoplasmic water activity	High	Very low
Enzymatic activity	Present	Absent
Macromolecular synthesis	Present	Absent
Heat resistance	Low	High
Resistance to chemicals , acids, radiation	Low	High

SHAPE AND POSITION OF ENDOSPORES

Shape:

usually smooth walled and ovoid in shape, but some are spherical and have ridges on wall

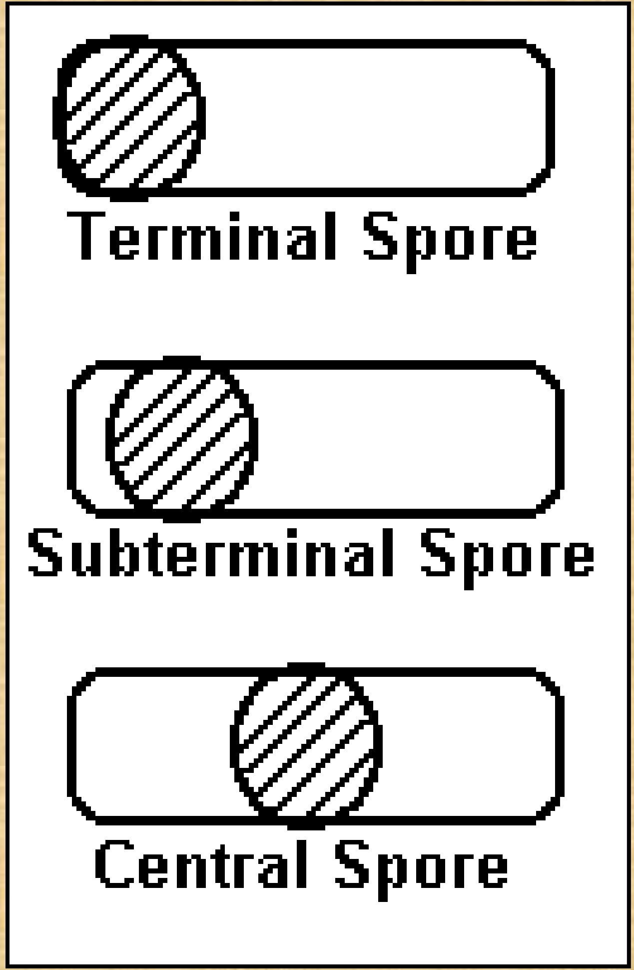
Size:

diameter of spore may be similar, more (bulging), or less than the vegetative cell

Position:

endospore, while attached to its parent cell, may be central, terminal, sub-terminal or lateral in position

The shape, size and position of an endospores are characteristic of a bacterial species giving it a particular shape, and is, therefore, of taxonomic value. For example, the spores of *Cl. tetani* are drum stick shaped and *Cl. botulinum* are tennis racquet shaped


Terminal Spore
Subterminal Spore
Central Spore

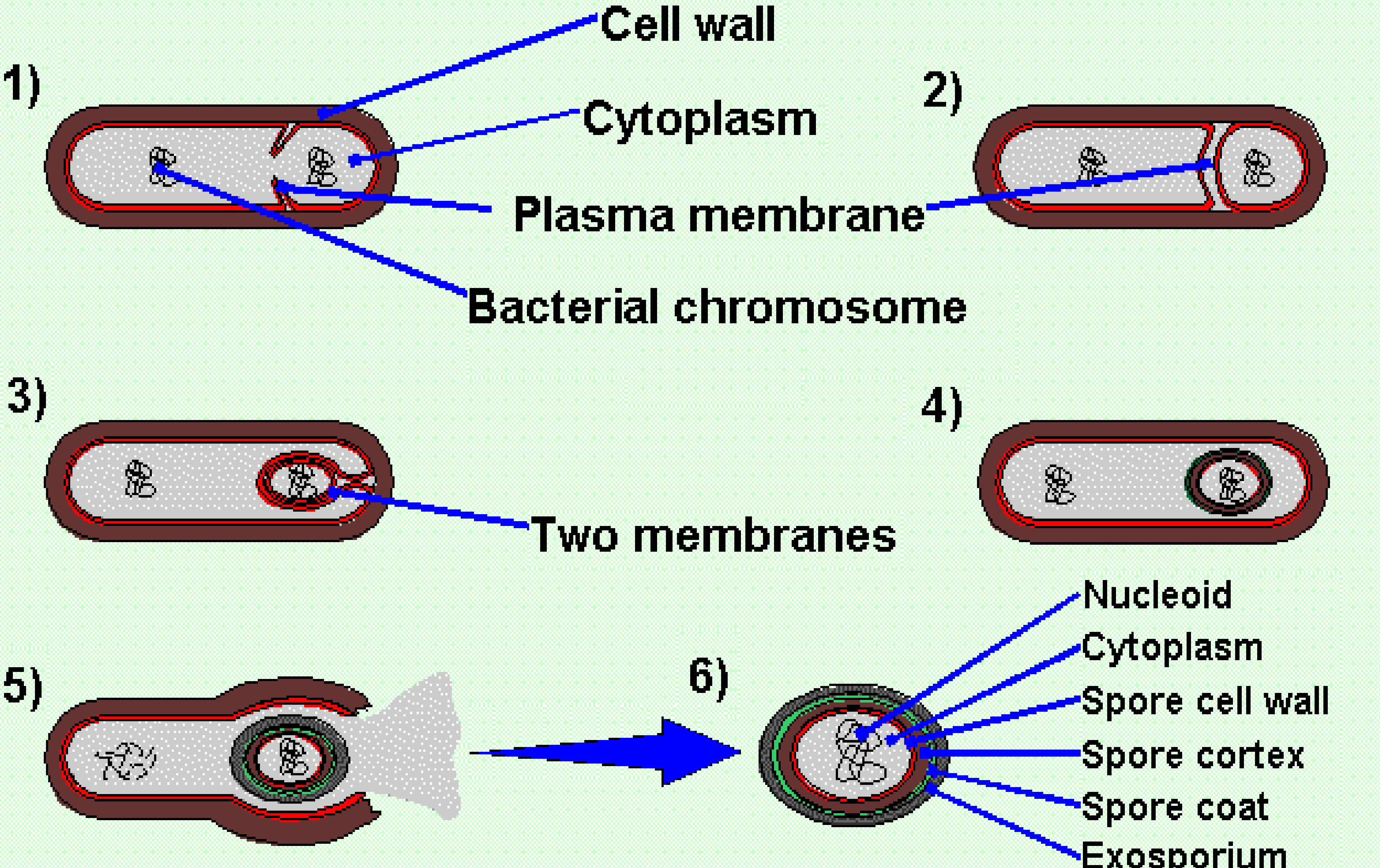
Bacterial endospores of *Clostridium tetani*

SPORULATION/SPOROGENESIS

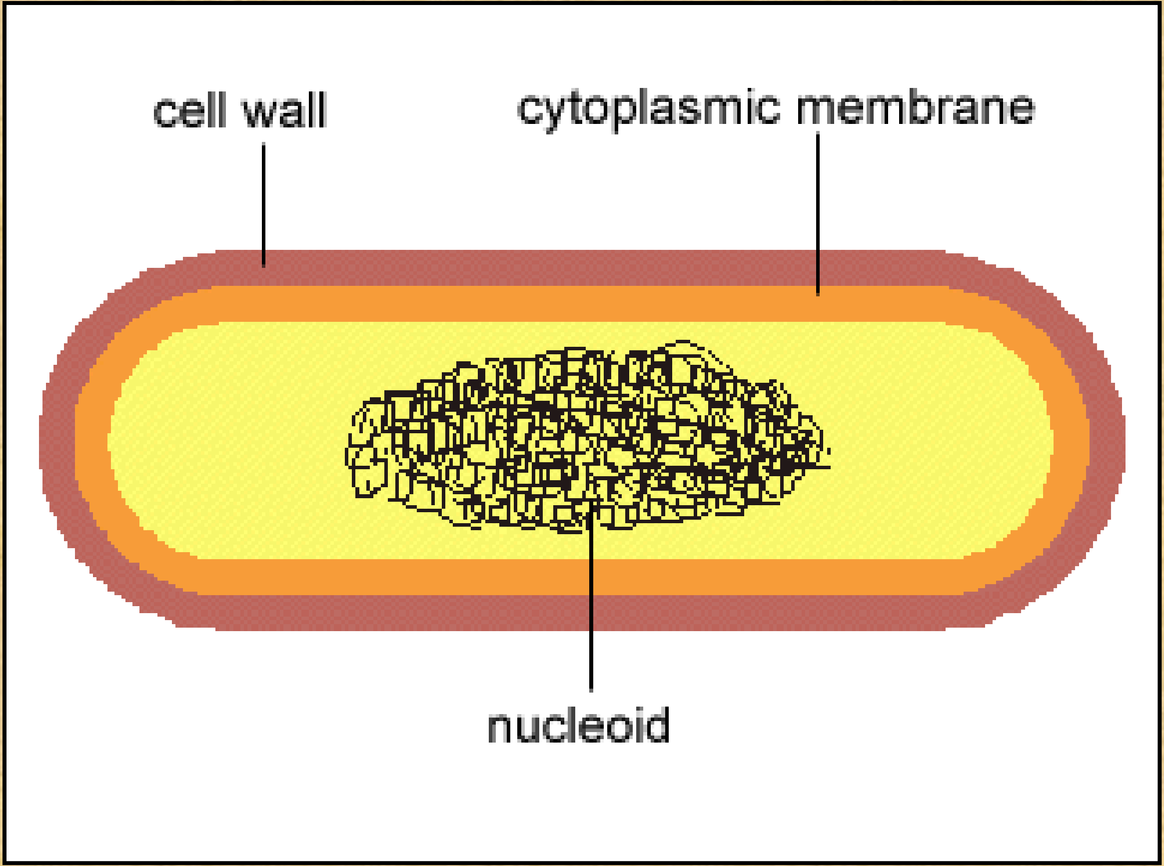
- **The process of formation of bacterial endospores is called as sporulation.**
- **Spore formation is usually triggered by adverse environmental growth conditions; usually limitation of carbon/nitrogen supply.**
- **Different biochemical events associated with sporulation are:**
  - **decreased in GTP and GDP,**
  - **accumulation of a novel purine metabolite - 3’5’-p_3Ap_3,**
  - **synthesis of Ca-dipicolinate, and**
  - **appearance of intracellular protease(s).**
- **Induction of sporulation leads to the derepression of many genes (about 200) encoding proteins necessary for spore formation.**
- **Sporulation generally takes around 15 hours.**

- **First the DNA replicates and condenses into an axial filament, which move towards one of the pole of bacterial cell.**
- **This triggers an asymmetrical cell division resulting in the invagination of plasma membrane into cytoplasm at one pole leading to the formation of a fore spore septum.**
- **The next step involves the outgrowth of the cell wall around the forespore protoplast resulting in the formation of a forespore surrounded by two membranes.**
- **A thick protective layer of mocified peptidoglycan called the cortex is synthesized between the inner and outer forespore membranes. Calcium dipicolinate is also synthesized and incorporated.**
- **Spore coat composed of a keratin-like protein then forms around the cortex.**
- **Sometimes an an exosporium is also formed**

Production of an endospore

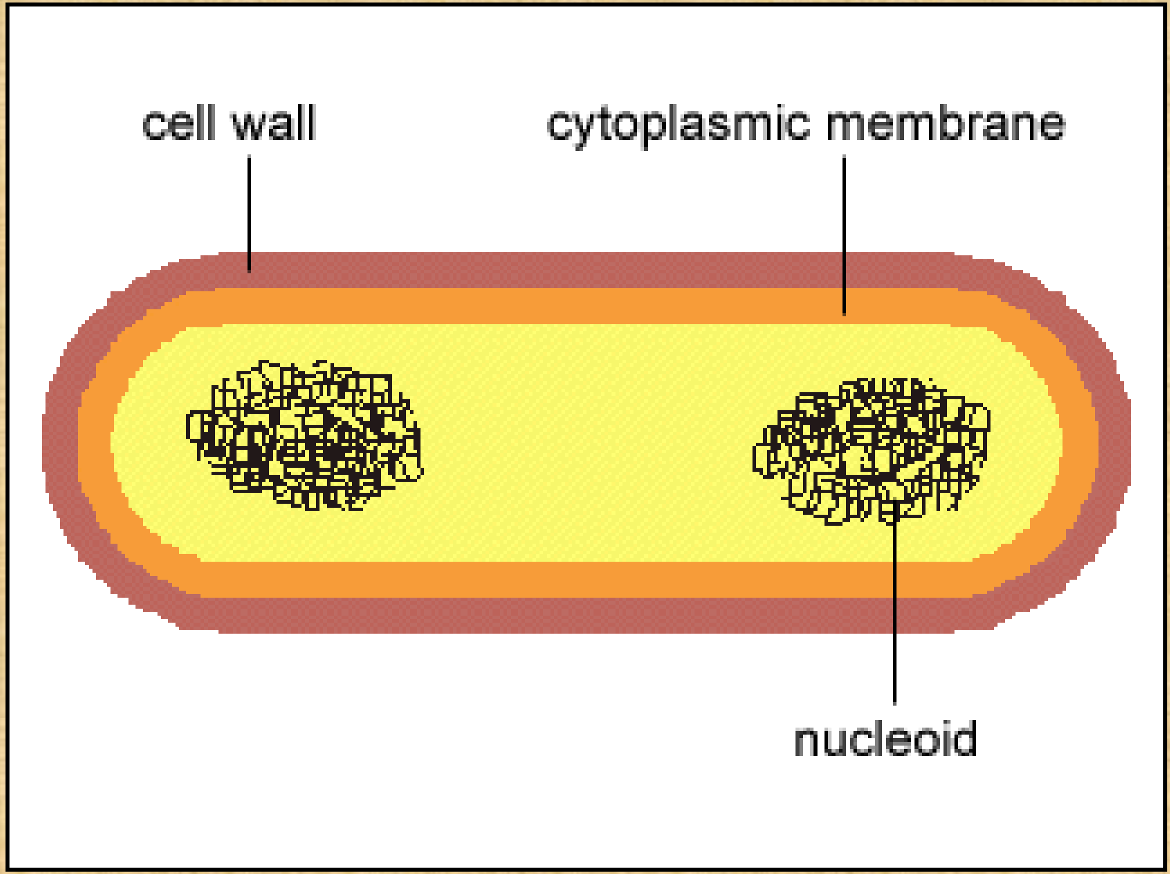


Endospore Cycle, Step 1



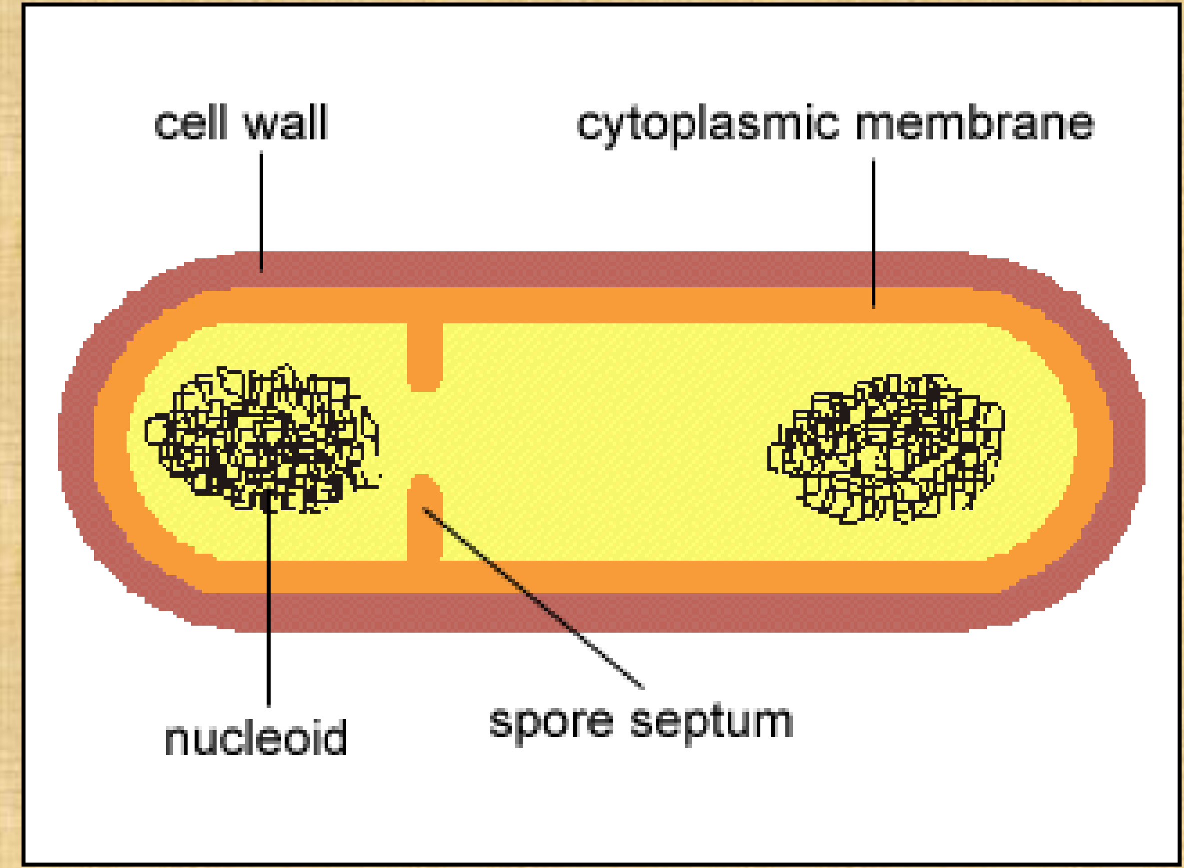
A vegetative bacterium about to enter the endospore cycle

Endospore Cycle, Step 2



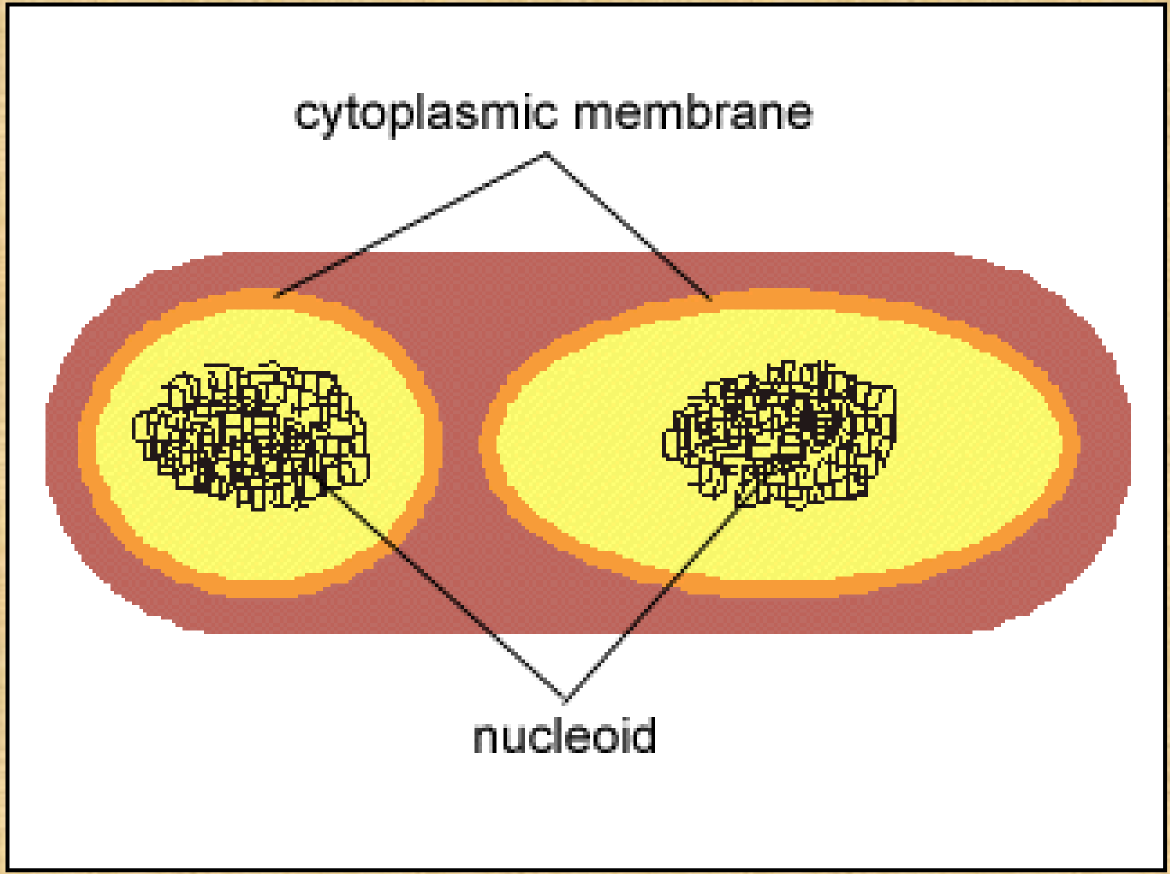
The nucleoid replicates

Endospore Cycle, Step 3



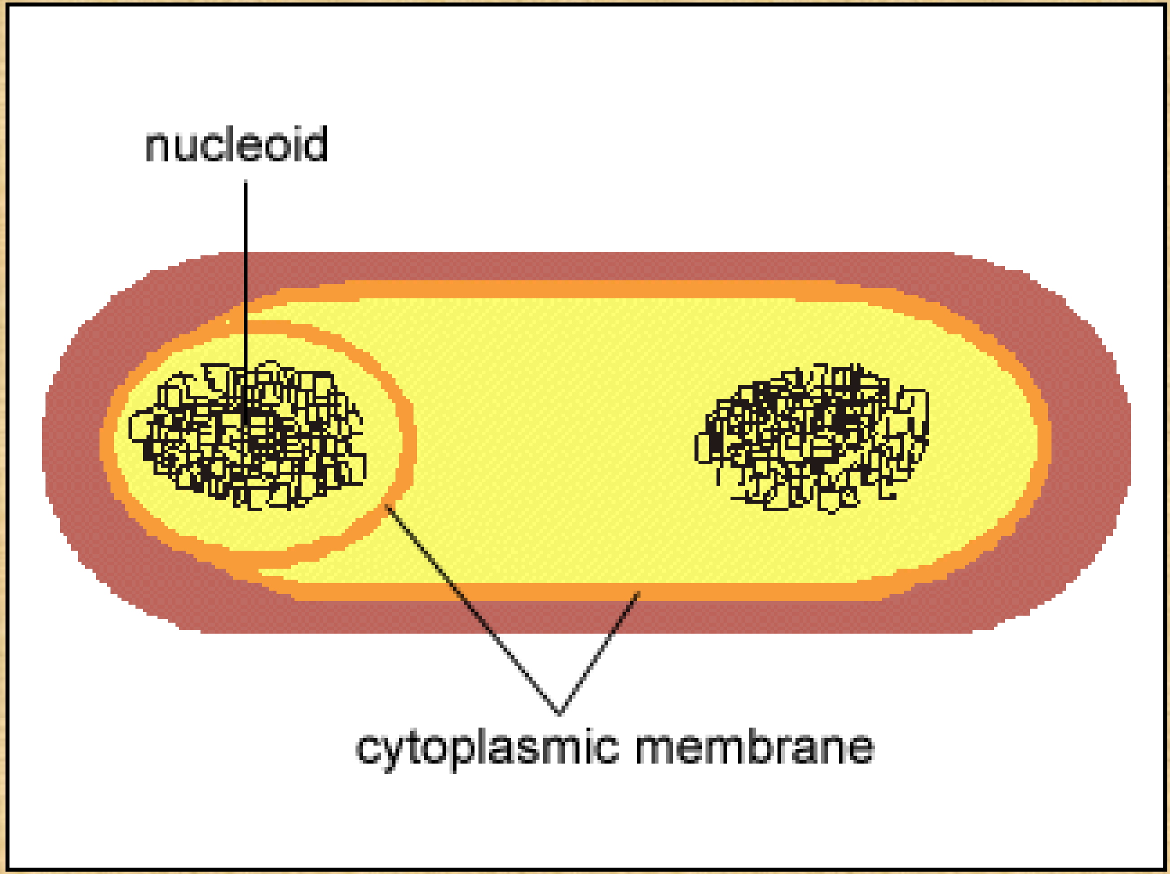
A fore spore septum forms

Endospore Cycle, Step 4



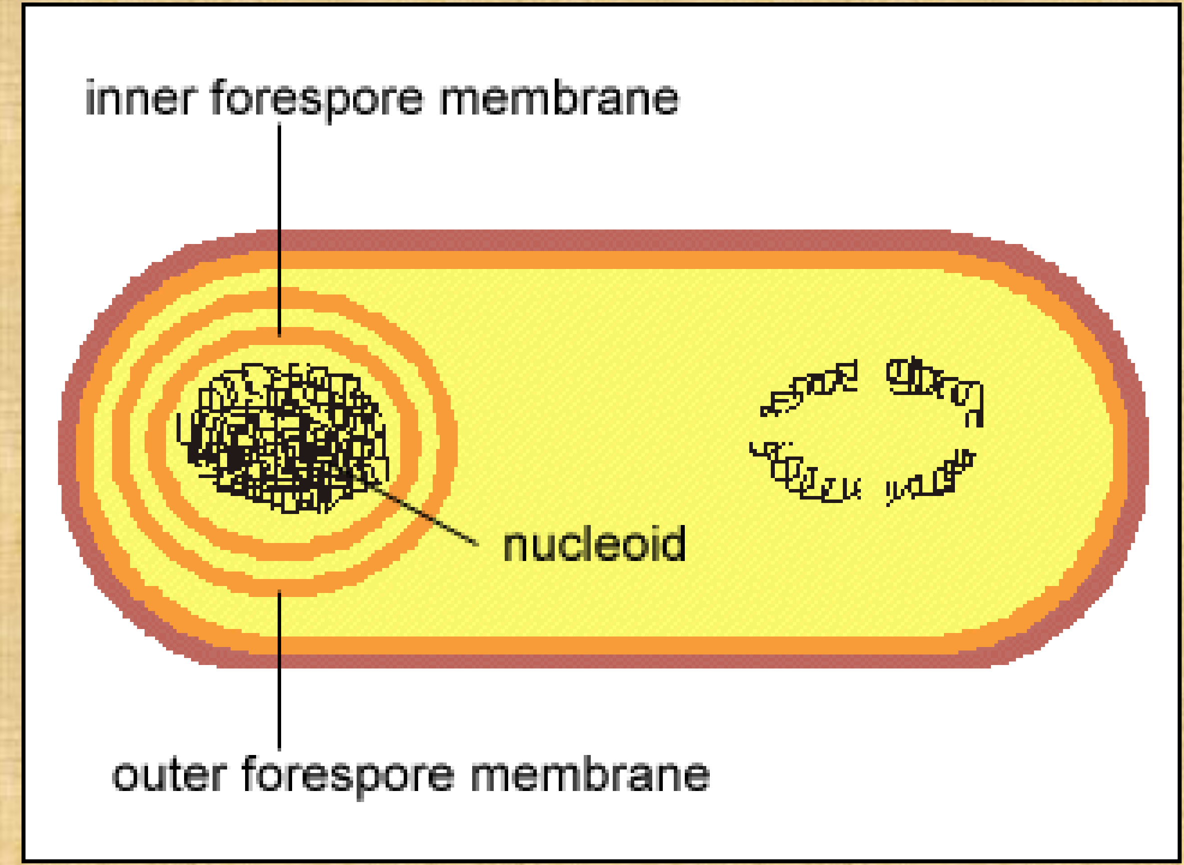
Each nucleoid becomes surrounded by its own cytoplasmic membrane.

Endospore Cycle, Step 5



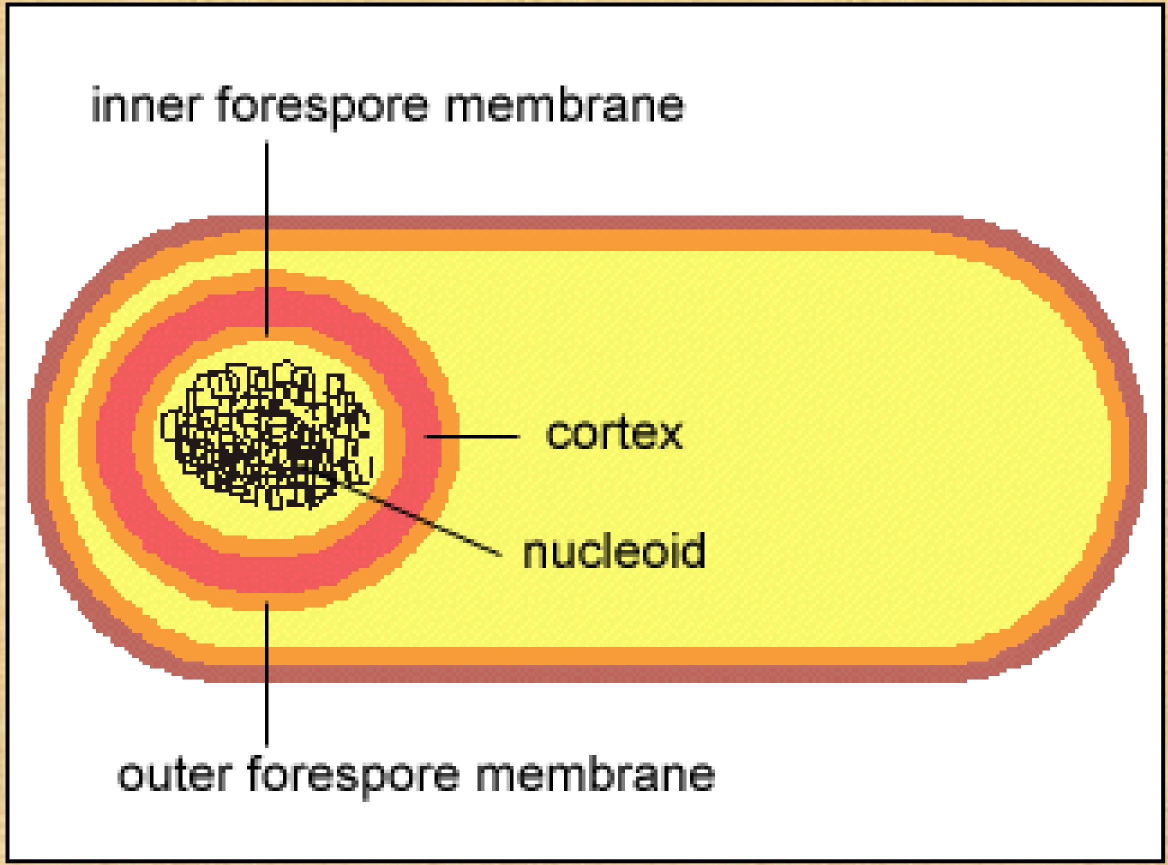
The cytoplasmic membrane surrounds the isolated nucleoid and cytoplasm forming a forespore

Endospore Cycle, Step 6



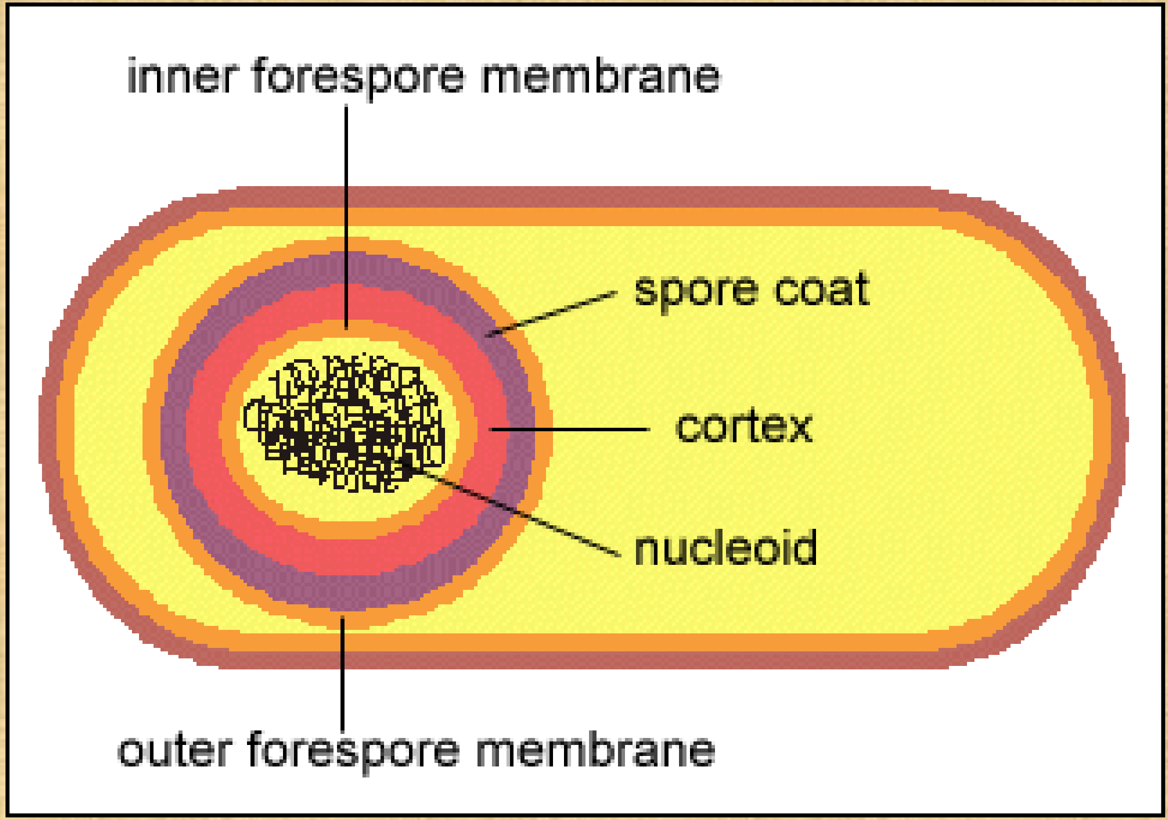
The forespore is completed and the other molecule of DNA is eventually degraded.

Endospore Cycle, Step 7



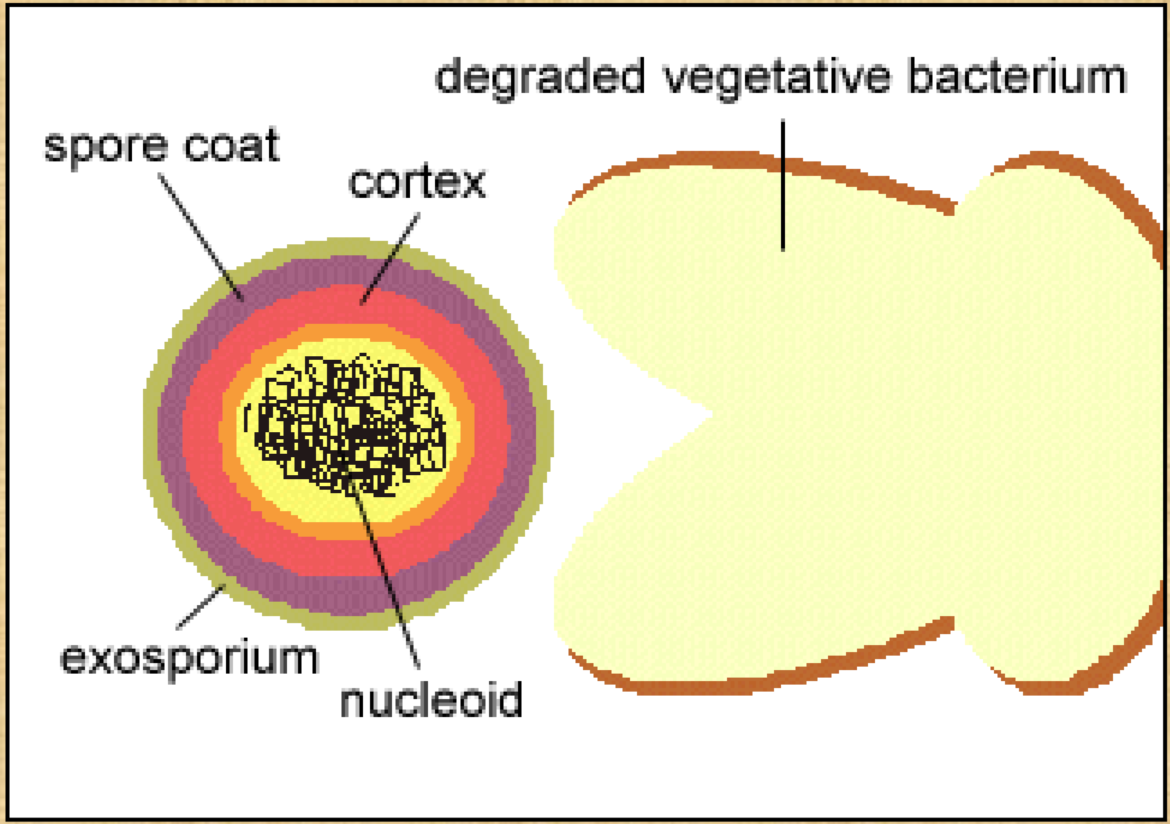
A thick protective layer of peptidoglycan called the cortex is synthesized between the inner and outer forespore membranes. Calcium dipicolinate is synthesized and incorporated in the forming endospore.

Endospore Cycle, Step 8



Another protective layer called the spore coat and composed of protein is synthesized.

Endospore Cycle, Step 9



Sometimes a final layer called the exosporium is added. As the vegetative portion of the bacterium is degraded, the completed endospore is released. Sporulation generally takes around 10-15 hours

GERMINATION OF SPORE

- **Under favorable environmental conditions and a suitable growth medium a spore 'germinate' to become a vegetative cell again.**

- **There are three recognizable stages in spore germination:**
  - **Activation**
  - **Germination**
  - **Outgrowth**

Activation

- **Prior to germination the spores undergo a so-called "activation" or maturation phase, which requires the exposure of the cells to high temperature or a prolonged 'resting period' (ageing).**
- **The percentage and rate of germination can be increased by heat, low pH or treatment with sulphydryl compounds, such as mercaptoethanol .**
- **No morphological changes have been demonstrated in this stage, but it appears that some conformational changes occur in the proteins of spore coat in this phase.**

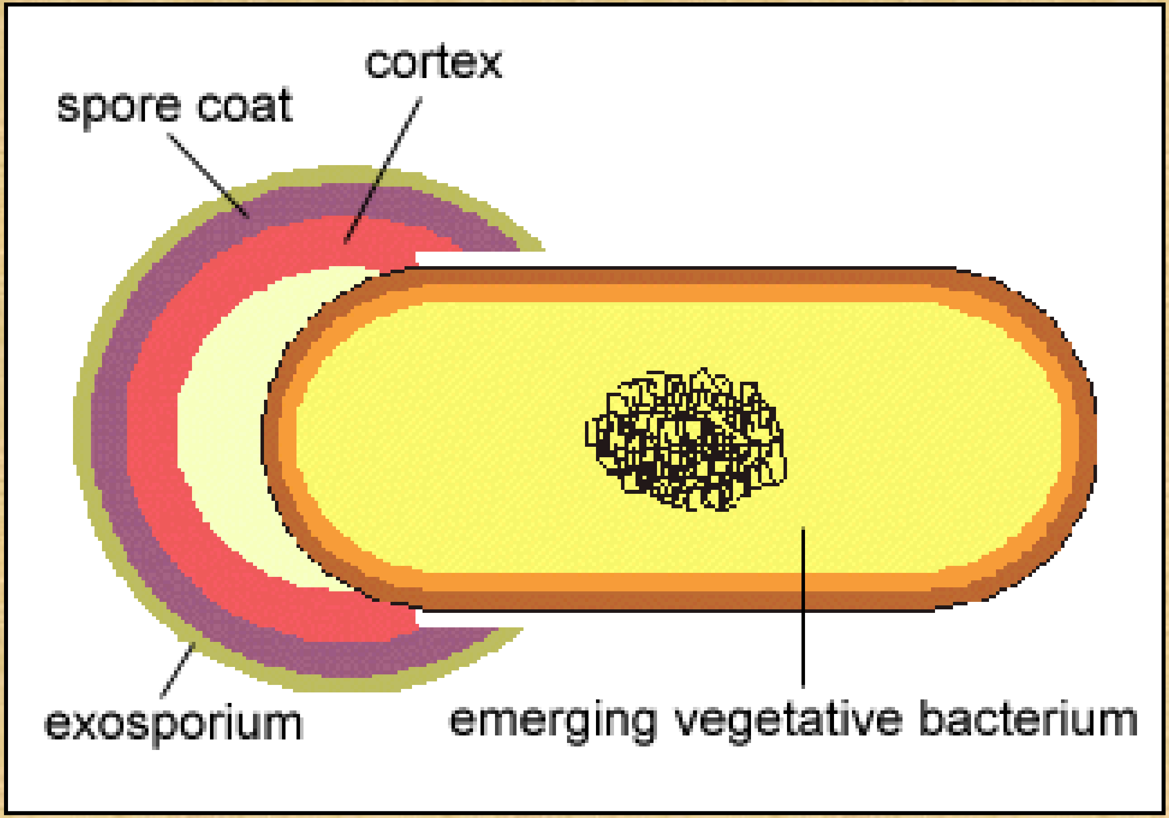
Germination

- **The germination of the spores initiated by water uptake and swelling causing damage to spore coat**
- **Germination requires a germination agent (glucose, amino acids).**
- **The agent penetrates the damaged spore coat and activates enzymes that hydrolyses the cortical peptidoglycan; its loose cross-linking and loss of Ca^{+2} ions promote digestion of peptidoglycan.**
- **During germination there is increased respiration and increase in metabolic activities.**
- **Germination specific proteases are formed that hydrolyses spore proteins to produce amino acids that are used to initiate protein synthesis in the vegetative cells.**
- **The high temperature resistance is lost due to loss of dipicolinic acid. Approximately 25-30% of the spore dry weight is lost. The entire process takes less than a minute.**

Outgrowth

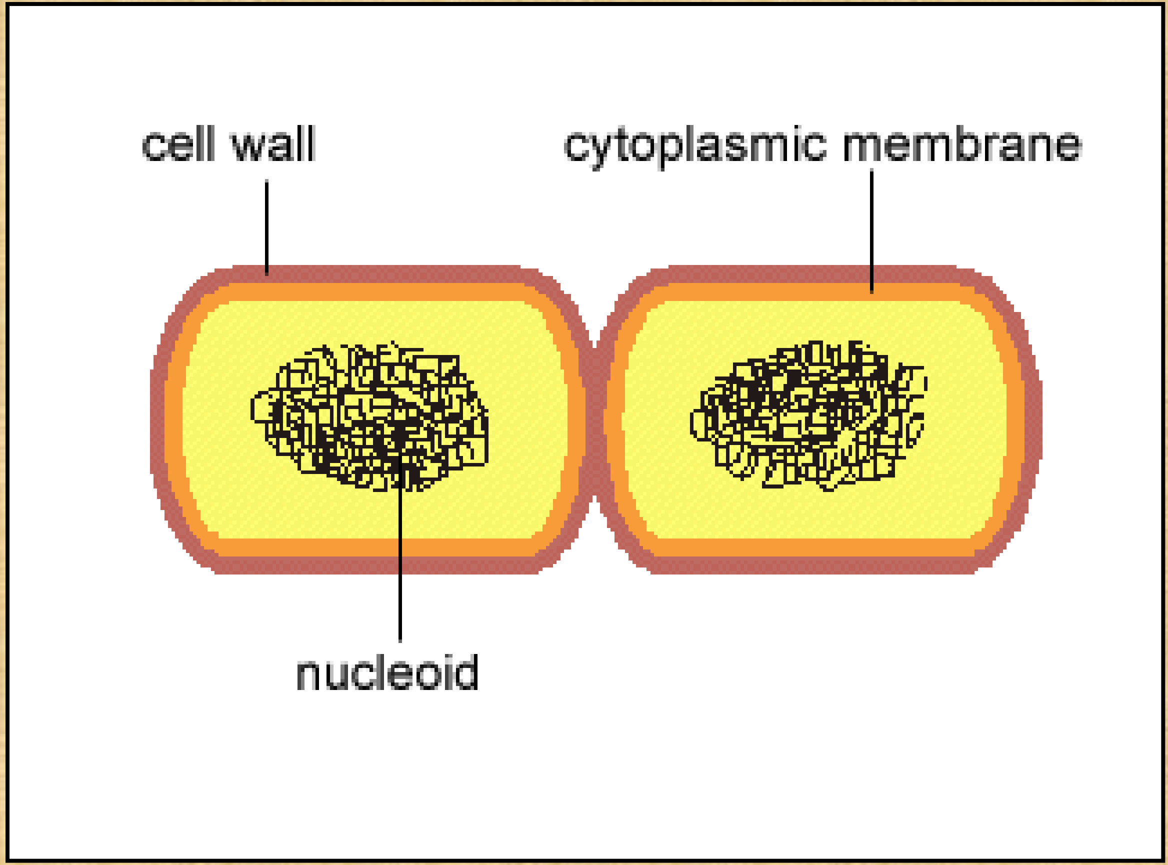
- The last stage involves the outgrowth of the vegetative cell leaving the spore coat behind.
- Outgrowth is defined as the stage from germination upto the formation of first vegetative cell and prior to the first binary fission.
- The first visible change in this stage is swelling of cell.
- A germination tube is then extruded either laterally (in *Bacillus*) or from the pole (in *Clostridia*) of spore.
- This is followed by completion of cell wall and in about an hour the synthesis of DNA starts.

Germination of Spores, Step 1



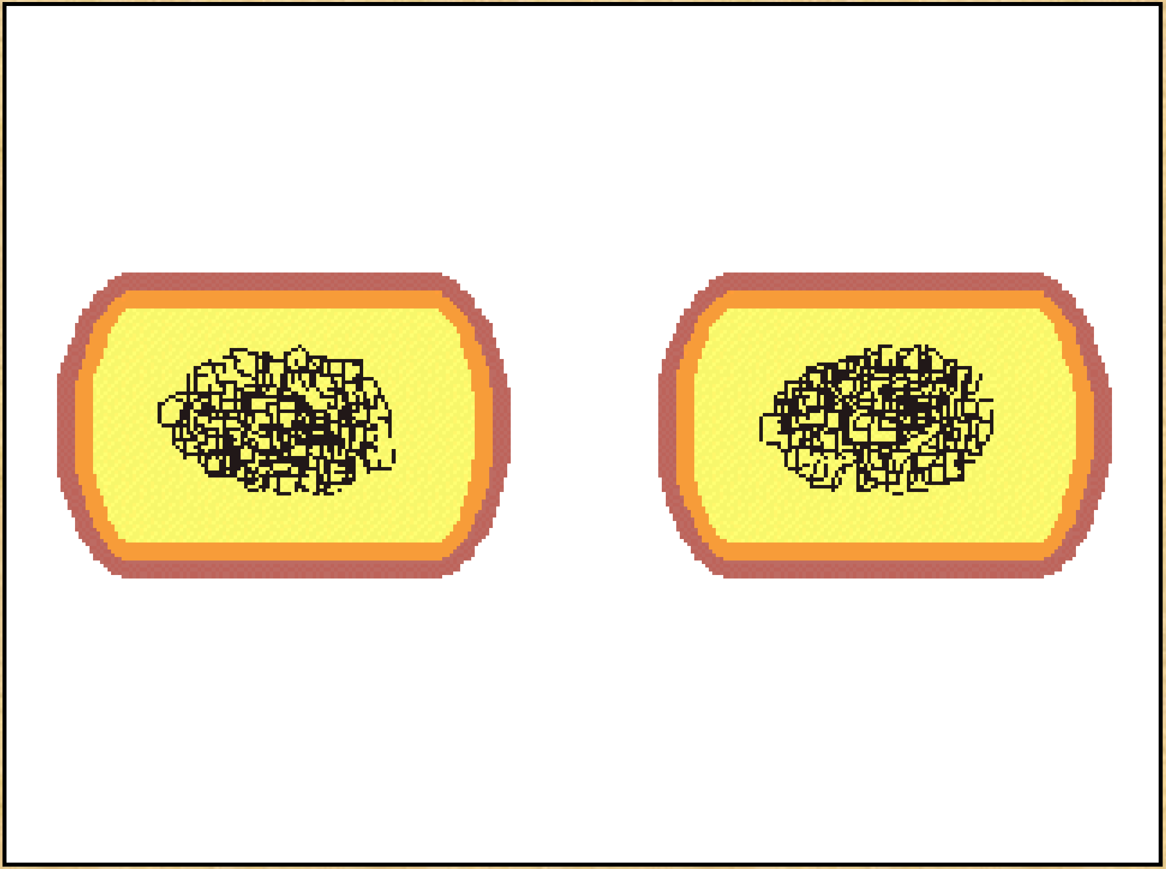
With the proper environmental stimuli, the endospore germinates. As the protective layers of the endospore are enzymatically broken down, a vegetative bacterium begins to form and emerge.

Germination of Spores, Step 2



The vegetative bacterium now begins to divide by binary fission.

Germination of Spores, Step 3



REASONS FOR RESISTANCE OF SPORES

- **Calcium-dipicolinate**
- **Specialized DNA-binding proteins**
- **Dehydration of spore cortex**
- **DNA repair enzymes**

DEMONSTRATION OF SPORES

- **Spores are normally impervious to stains.**
- **Under the light microscope endospores have a high light refractivity indicative of high protein content.**
- **Selective spore stains can enhance the spore structure and more detailed structures are revealed by studies under the electron microscope.**
- **Endospores can be stained by modified Zeihl-Nelson's method using 0.25-0.5% sulphuric acid as decolorizing agent, Barthelomew-Mittwar’s method or Schaeffer-Fulton stain technique.**

Exospores

Exospores are desiccation and heat resistant spores produced by a budding process in members of the genus *Metylosinus* and *Rhodomicrobium*. These do not contain dipicolinic acid.

Condiospores and sporangiospores

Actinomycetes form branching hyphal spores that develop singly or in chains from the tips of hyphae by cross wall formation. If these spores are enclosed in a sac they are called sporangiospores; if not they are called conidiospores. These spores do not have heat resistance but they can survive for longer period of drying.

Cysts

Cysts are dormant, thick-walled, desiccation resistant forms that develop by differentiation of an entire vegetative cell which can later germinate under suitable conditions. The cyst itself confers resistance to desiccation, radiation and mechanical stress but not to high temperatures. These are produced by genus *Azotobacter*.