

Bacterial Endospore

By – Dr. Ekta Khare

Endospores

- A number of gram-positive bacteria can form a special resistant, dormant structure called an **endospore**.
- An endospore is a dormant, tough, and non-reproductive structure.
- The name "endospore" is suggestive of a spore or seed-like form, but it is not a true spore (i.e., not an offspring). It is a stripped-down, dormant form to which the bacterium can reduce itself.
- Endospores develop within vegetative bacterial cells of several genera: *Bacillus* and *Clostridium* (rods), *Sporosarcina* (cocci), and others.
- These structures are extraordinarily resistant to environmental stresses such as:
 - heat,
 - ultraviolet radiation,
 - gamma radiation,
 - chemical disinfectants, and
 - desiccation
- Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance in food, industrial, and medical microbiology.
- There are many reports of spores remaining viable over 10,000 years, and revival of spores millions of years old has been claimed. There is one report of viable spores of *Bacillus marismortui* in salt crystals approximately 250 million years old.

- Because of their resistance and the fact that several species of endospore-forming bacteria are dangerous pathogens, endospores are of great practical importance in food, industrial, and medical microbiology.
- This is because it is essential to be able to sterilize solutions and solid objects.
- Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials.
- Endospores can be examined with both light and electron microscopes.
- Because spores are impermeable to most stains, they often are seen as colorless areas in bacteria treated with methylene blue and other simple stains; special spore stains are used to make them clearly visible.
- Spore position in the mother cell or sporangium frequently differs among species, making it of considerable value in identification.
- Spores may be centrally located, close to one end (subterminal), or definitely terminal (figure 3.40).
- Sometimes a spore is so large that it swells the sporangium.

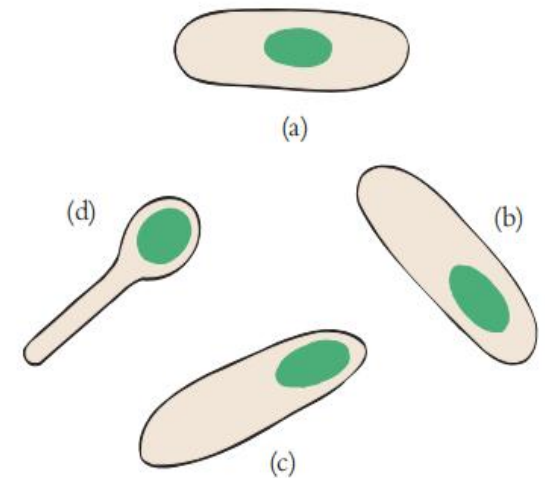
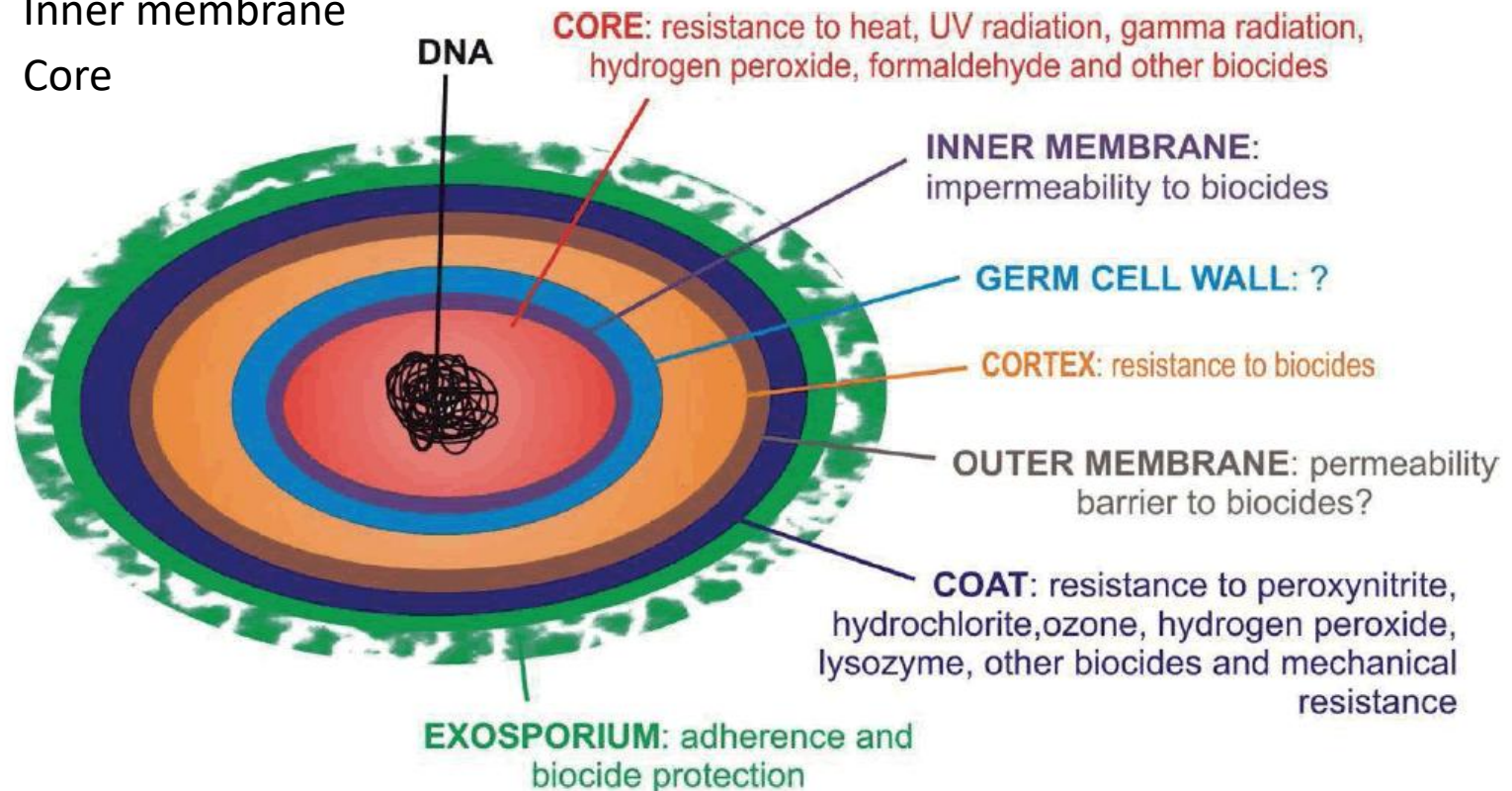


Figure 3.40 Examples of Endospore Location and Size. (a) Central spore. (b) Subterminal spore. (c) Terminal spore. (d) Terminal spore with swollen sporangium.

Endospore Structure

- An endospore has following layers:
 - Exosporium
 - Spore coat
 - Outer membrane
 - Cortex
 - Spore cell wall or core wall or germ cell wall
 - Inner membrane
 - Core



Exosporium:

- It is the outermost layer made up of protein that encloses spore coat.
- In some bacterial spore, exosporium is made up of polysaccharide and lipid.

Spore coat:

- It is thick double layered covering that encloses outer membrane.
- Spore coat consists of spore specific protein, mainly contains cysteine and hydrophobic amino acids. Due to presence of these amino acids, spore are resistant to adverse environmental condition.

Outer membrane:

- The precise function of the outer membrane that lies under the spore coats is not clear, although this membrane is an essential structure in spore formation.

Cortex:

- Cortex made up of loosely arranged peptidoglycan layer.
- Inner layer: comprises about 20% of peptidoglycan, it is tightly arranged
- Outer layer: it is loosely arranged, it can be hydrolysed during spore germination. It comprises alanine (55%), tetra-peptide (15%) and muramic lactum (30%).

Spore cell wall/ core wall, germ cell wall:

- Present inside the cortex and surrounds the protoplast or core, made up of peptidoglycan.

Inner membrane:

- The inner spore membrane is a strong permeability barrier that plays a major role in spore resistance to many chemicals, in particular those that could damage the spore DNA

Core:

- It is the innermost part of spore, also known as spore protoplast.
- Core consists of cytoplasm, nucleoid, ribosomes and other cellular materials surrounded by inner or cytoplasmic membrane.
- Core contains (10-25%) water so, the cytoplasm is gel like.
- It contains high amount of calcium and dipicolinic acid as calcium dipicolinate (CaDPA) (10-15% by dry weight).
- Core also contains high percentage of small acid soluble protein (SASP).
- SASP is synthesized during sporulation and it binds to DNA in core and protect it from potential damage caused by UV radiation, desiccation and drying.
- In addition, SASPs also provides nutrition and energy for spore germination.
- Core also contain some DNA repair enzymes. DNA is repaired during germination and outgrowth after the core has become active once again.

In summary, endospore heat resistance probably is due to several factors: calcium-dipicolinate and acid-soluble protein stabilization of DNA, protoplast dehydration, the spore coat, DNA repair, the greater stability of cell proteins in bacteria adapted to growth at high temperatures, and others.

Sporogenesis/ sporulation

- Spore formation, sporogenesis or sporulation, normally commences when growth ceases due to lack of nutrients.
- It is a complex process and may be divided into seven stages.

Stage 0

- Normal conditions of a vegetative cell

Stage I: Axial filament formation stage

- In this stage bacterial chromosome become thread like known as axial filament
- Axial filaments attached to cytoplasmic membrane by mesosome
- Elongation of cell take places
- PHB is the reserved food material in *Bacillus spp* is utilized in sporulation.

Stage II: forespore formation

- Asymmetric cell division occurs
- Cell membrane forms septum near one end which encloses a small portion of DNA forming forespore

Stage III: engulfment of forespore

- Mother cell membrane grow around the forespore engulfing it.
- Fore spore now has two membrane layer.

Stage IV: synthesis of exosporium

- Chromosome of mother cell disintegrates
- Exosporium synthesis occurs
- Forespore starts Forming primordial cortex between two membrane.
- Dehydration of cell

Stage V: synthesis of dipicolonic acid

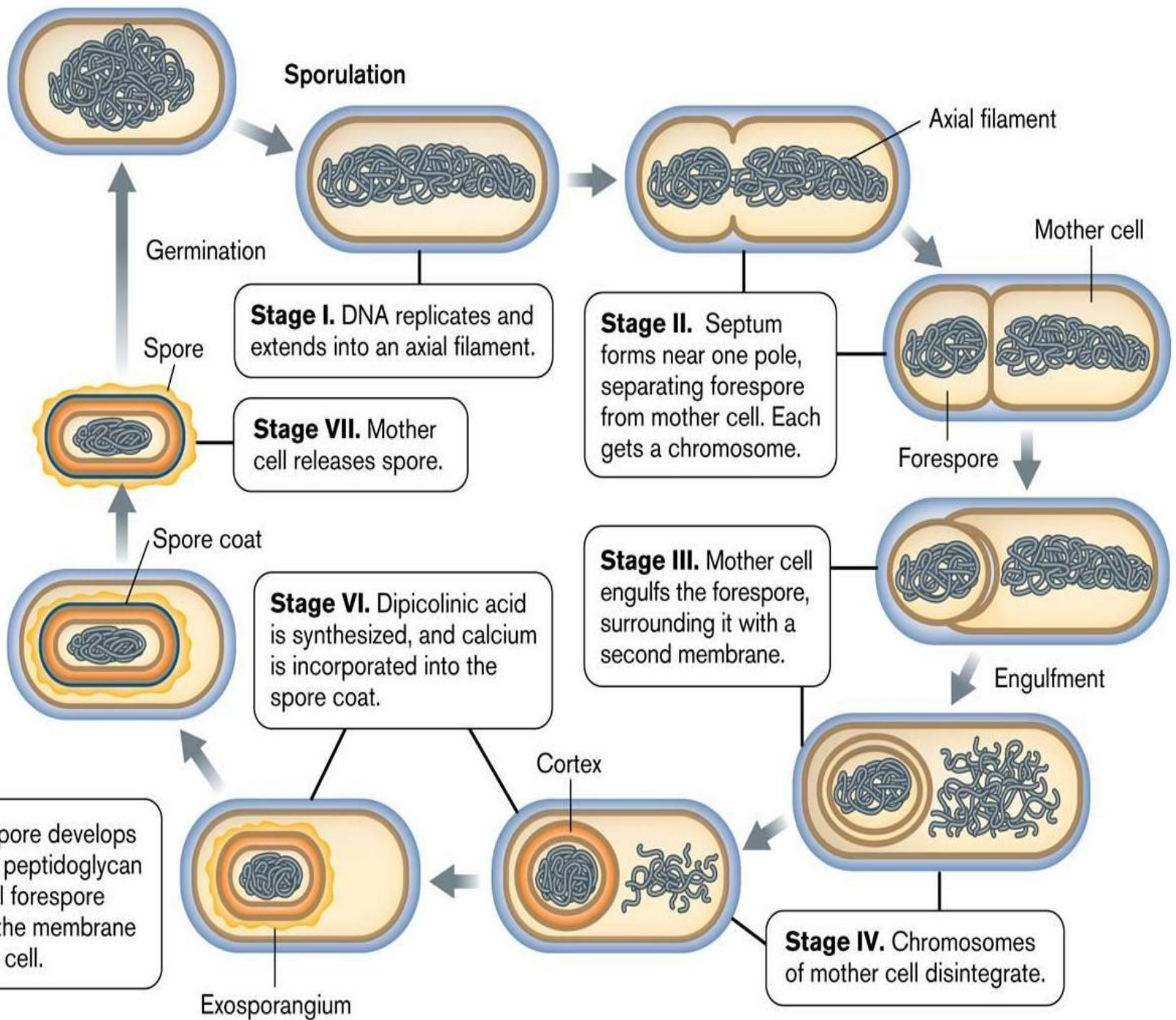
- Production of SASPs and dipicolinic acid occurs
- Incorporation of calcium ions with dipicolonic acid occur forming calcium dipicolonate
- Further dehydration of cytoplasm
- Formation of coat layer

Stage VI: maturation

- Maturation of endospore

Stage VII: release of endospore

- Lytic enzymes destroy the sporangium releasing the spore



Endospore germination

- Germination is triggered by environmental stimuli that are then transduced into a series of interrelated degradation events. This ultimately results in the loss of typical dormant endospore properties.
- The transformation of dormant spores into active vegetative cells occurs in three stages: (1) activation, (2) germination, and (3) outgrowth.

Activation:

- There are a number of different types of agents that trigger spore germination, and some of these, such as CaDPA and cationic surfactants like dodecylamine, and high temperature, probably are important only in the laboratory.
- However, in nature, it is likely that the presence of specific nutrients is what triggers spore germination.
- The available evidence is consistent with nutrient germinants binding in a stereospecific manner to spore-specific protein complexes, termed germinant receptors (GRs), in the spore membrane.
- Once triggered, the germination process continues in the absence of nutrients, a finding that indicates that germination is controlled by the sequential activation of preexisting enzymes and does not require protein synthesis.

Germination:

- After germinant receptor activation, the endospore's core is rehydrated and cations from the endospore core, including the large depot of CaDPA complex, are released. Even now, little is known about the identities and functions of the proteins that mediate these fluxes.
- CaDPA release completes stage I of germination and also triggers entry into stage II, when cortex lytic enzymes (CLEs) degrade the peptidoglycan cortex.
- Another major process that occurs during II stage of germination is the breakdown of endospore-specific proteins (e.g. SASPs) and structures (e.g. cortex).
- The metabolites released during these degradation processes are believed to serve as raw materials for the developing cell.
- Some endospores can also use released amino acids as quorum sensing signals to synchronize entry into vegetative growth.

Out growth:

- Cells take their usual rod-shape and get ready to divide .
- Since endospores are devoid of mRNA, outgrowth is dependent on de novo transcription and translation. mRNA synthesis begins in the first minutes of germination and precedes protein synthesis by several minutes.
- DNA synthesis does not start until 30 minutes after germination onset when chromosome replication begins.

Stage I

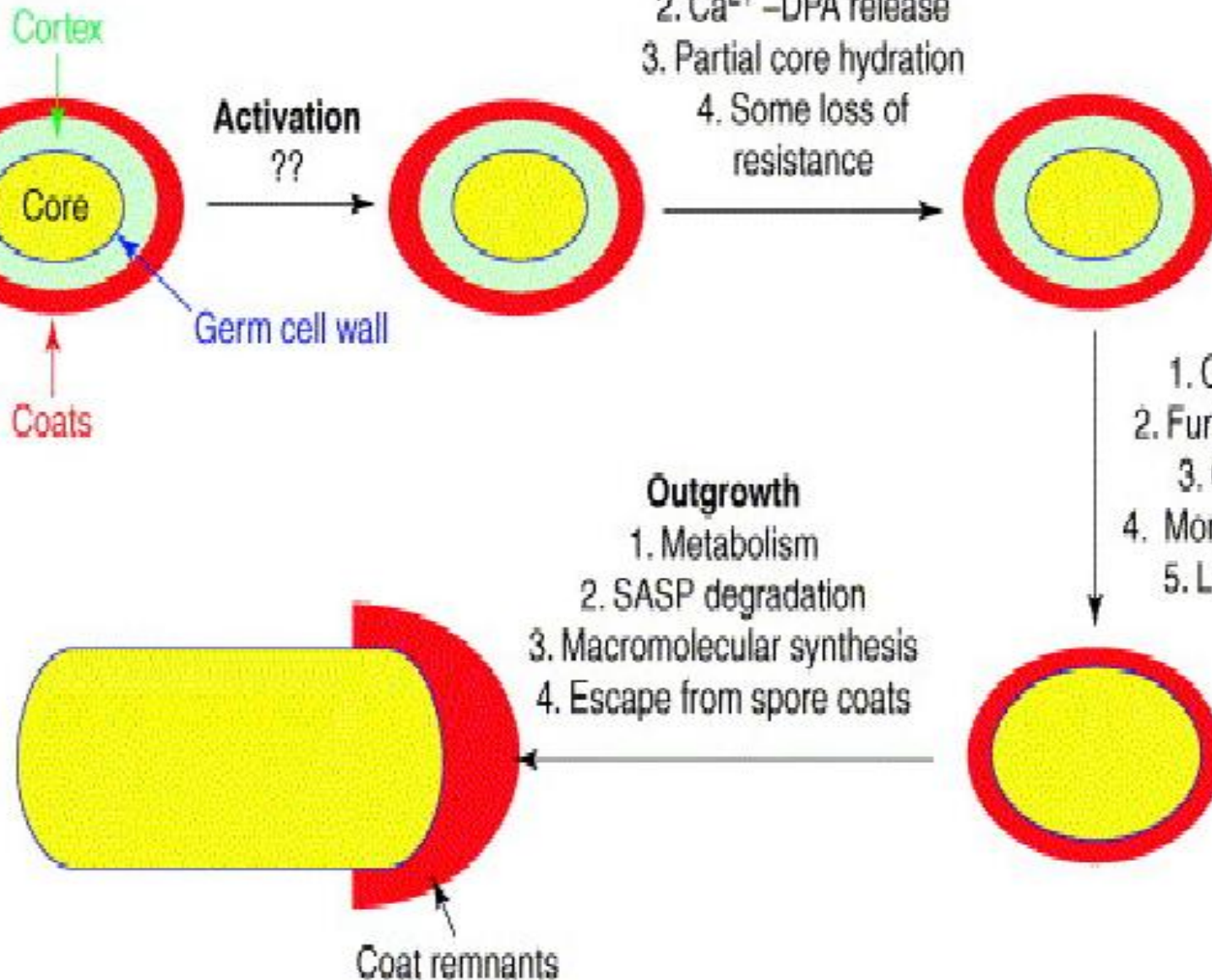
1. Cation release
2. Ca^{2+} -DPA release
3. Partial core hydration
4. Some loss of resistance

Stage II

1. Cortex hydrolysis
2. Further core hydration
3. Core expansion
4. More loss of resistance
5. Loss of dormancy

Outgrowth

1. Metabolism
2. SASP degradation
3. Macromolecular synthesis
4. Escape from spore coats



Questions

- Explain sporogenesis in detail. What are the events involve in endospore germination?
- Explain sporogenesis giving only diagram.
- Write short note on endospore germination.
- Discuss structure of endospore. What are the component responsible for endospore tolerance to harsh environment conditions.