Bacterial endospore

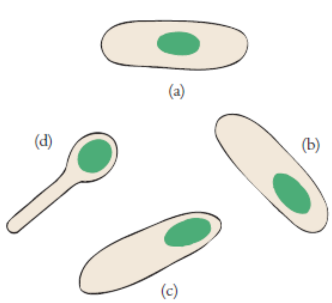
A number of gram positive bacteria can form a special resistant, dormant structure called endospore.Endospores develop within vegetative bacterial cells of several genera: *Bacillus* and *Clostridium* (rods), *Sporosarcina* (cocci), and others. Bacteria forms spores under adverse environmental conditions such as limited supply of nutrients, heat, drying, freezing, exposure to toxic chemicals and radiations. Endospores are extraordinarily resistant to environmental stress such as heat, ultraviolet radiation, gamma radiation, chemical disinfectants and desiccation.

Spore forming bacteria

The two medically-important genera are *Bacillus*, the members of which areaerobic spore formers in the soils, and *Clostridium*, whose species are anaerobic spore formers of soils, sediments and the intestinal tracts of animals.Some spore formers are pathogens of animals, usually due to the production of powerful toxins. *Bacillus anthracis* causes anthrax, a disease of domestic animals. *Bacillus cereus* causes food poisoning. *Clostridium botulinum* causes botulism, a form of food poisoning, and *Clostridium tetani* is the agent of tetanus. *Clostridiumperfringens* causes food poisoning, anaerobic wound infections and gas gangrene, and *Clostridium difficile* causes a severe form of colitis called pseudomembranous colitis.

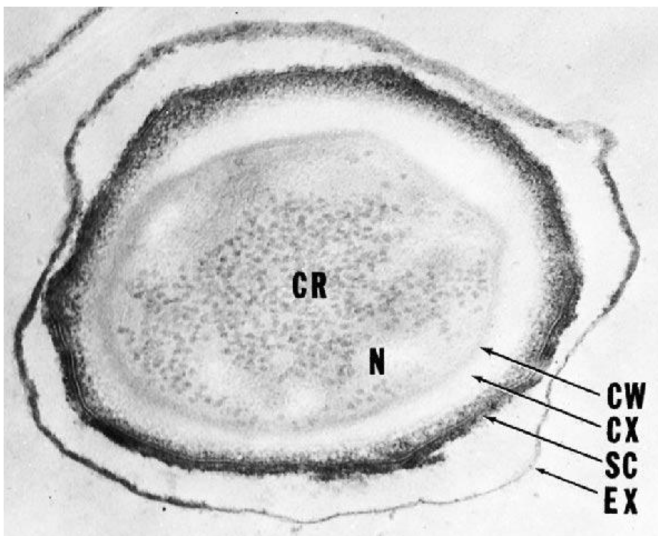
Properties

Endospores can be examined with both light and electron microscopes. They can be observed with the phase-contrast microscope or negative staining. Endospores are not stained well by most dyes, but once stained, they strongly resist decolorization. This property is the basis of most spore staining methods. In the Schaeffer-Fulton procedure, endospores are first stained by heating bacteria with malachite green, which is a very strong stain that can penetrate endospores. After malachite green treatment, the rest of the cell is washed free of dye with water and is counterstained with safranin. This technique yields a green endospore resting in a pink to red cell.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. Sometimes a spore is so large that it swells the sporangium.



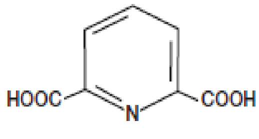
Structure

Electron micrographs show that endospore structure is complex . The spore often is surrounded by a thin, delicate covering called the exosporium. A spore coat lies beneath the exosporium, is composed of several protein layers, and may be fairly thick. It is impermeable and responsible for the spore's resistance to chemicals. The cortex, which may occupy as much as half the spore volume, rests beneath the spore coat. It is made of a peptidoglycan that is less cross-linked than that in vegetative cells. The spore cell wall (or core wall) is inside the cortex and surrounds the protoplast or core. The core has the normal cell structures such as ribosomes and a nucleoid, but is metabolically inactive.



Endospore Structure: Bacillus anthracis endospore,EX; spore coat, SC; cortex, CX; core wall, CW; and the protoplast or core with its nucleoid, N, and ribosomes, CR.

It is still not known precisely why the endospore is so resistant to heat and other lethal agents. As much as 15% of the spore's dry weight consists of dipicolinic acid complexed with calcium ions , which is located in the core. It has long been thought that dipicolinic acid was directly involved in spore heat resistance, but heat-resistant mutants lacking dipicolinic acid now have been isolated.



Dipicolinic Acid.

Calcium does aid in resistance to wet heat, oxidizing agents, and sometimes dry heat. It may be that calcium-dipicolinate often stabilizes spore nucleic acids. Recently specialized small, acid-soluble DNA-binding proteins have been discovered in the endospore. They saturate spore DNA and protect it from heat, radiation, dessication, and chemicals. Dehydration of the protoplast appears to be very important in heat resistance. The cortex may osmotically remove water from the protoplast, thereby protecting it from both heat and radiation damage. The spore coat also seems to protect against enzymes and chemicals such as hydrogen peroxide. Finally, spores 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. Endospores often survive boiling for an hour or more; therefore autoclaves must be used to sterilize many materials.

Sporogenesis

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.

An axial filament of nuclear material forms (stage I), followed by an inward folding of the cell membrane to enclose part of the DNA and produce the forespore septum (stage II). The membrane continues to grow and engulfs the immature spore in a second membrane (stage III).

Next, cortex is laid down in thespace between the two membranes, and both calcium and dipicolinic acid are accumulated (stage IV). Protein coats then are formed around the cortex (stage V), and maturation of the spore occurs (stage VI). Finally, lytic enzymes destroy the sporangium releasing the spore (stage VII). Sporulation requires only about 10 hours in *Bacillus megaterium*.When *B. subtilis* is deprived of nutrients, it will form endospores in a complex developmental process lasting about 8 hours.

* centrally located : central.

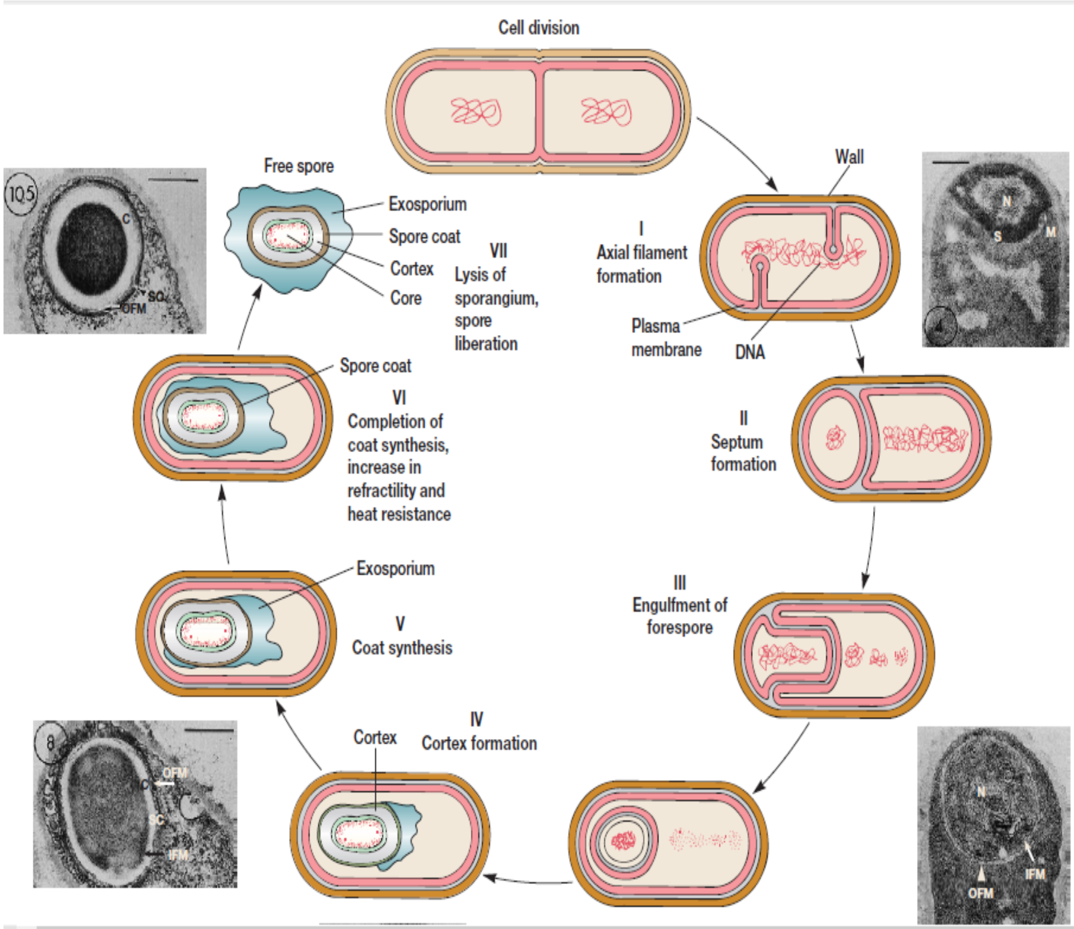
Eg.,*Bacillus cereus*

* close to one end : sub terminal.

Eg., *Bacillus subtilis*

* definitely terminal : terminal.

Eg.,*Clostridium tetani*



Germination

The transformation of dormant spores into active vegetative cells seems almost as complex a process as sporogenesis. It occurs in three stages: (1) activation, (2) germination, and (3) outgrowth. Often an endospore will not germinate successfully, even in a nutrient-rich medium, unless it has been activated. Activation is a reversible process that prepares spores for germination and usually results from treatments like heating. It is followed by germination, the breaking of the spore's dormant state. This process is characterized by spore swelling, rupture or absorption of the spore coat, loss of resistance to heat and other stresses, loss of refractility, release of spore components, and increase in metabolic activity. Many normal metabolites or nutrients (e.g., amino acids and sugars) can trigger germination after activation. Germination is followed by the third stage, outgrowth. The spore protoplast makes new components, emerges from the remains of the spore coat, and develops again into an active bacterium.

REFERENCE:

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