ESCOLA DE ENGENHARIA DE LORENA - USP
CURSO ENGENHARIA BIOQUÍMICA
DISCIPLINA MICROBIOLOGIA GERAL - 2015
3/09 / 2015)

Profa. Dra Bernadete Medeiros

OS GÊNEROS DE BACTÉRIAS FORMADORAS DE ENDOSPOROS

TABELA 1. GÊNEROS DE BACTÉRIAS FORMADORAS DE ENDOSPOROS

GÊNERO	G + C (mol %)	CARACTERÍSTICAS METABÓLICAS
Bacillus	33-66	Aeróbio estrito e catalase positivo
Clostridium	24-54	Anaeróbio estrito
Desulfotomaculum	37-50	Anaeróbia e Redutora Sulfato
Thermoactinomycetes	52-55	Aeróbia estrito
Sporosarcina	40-42	Aeróbio estrito e Urease positivo
Sporosarcina halophila		Halófilos
Sporolactobacillus	38-40	Fermentação homolática e anaeróbio facultativo ou microaerófilos
Heliobactyerium		Fototróficos

ATENÇÃO: AS ESPÉCIES QUE ESPORULAM SÃO BACTÉRIAS PROVENIENTES DE HABITATS DIVERSOS, HETEROGÊNEAS QUANTO AO METABOLISMO E NÃO ESTÃO RELACIONADAS QUANTO A FILOGENIA.

% GC = G+C / A+T+C+G x 100

A,T,C,G são as concentrações molares de adenina, timina, citosina e guanina

CICLO DE ESPORULAÇÃO E GERMINAÇÃO

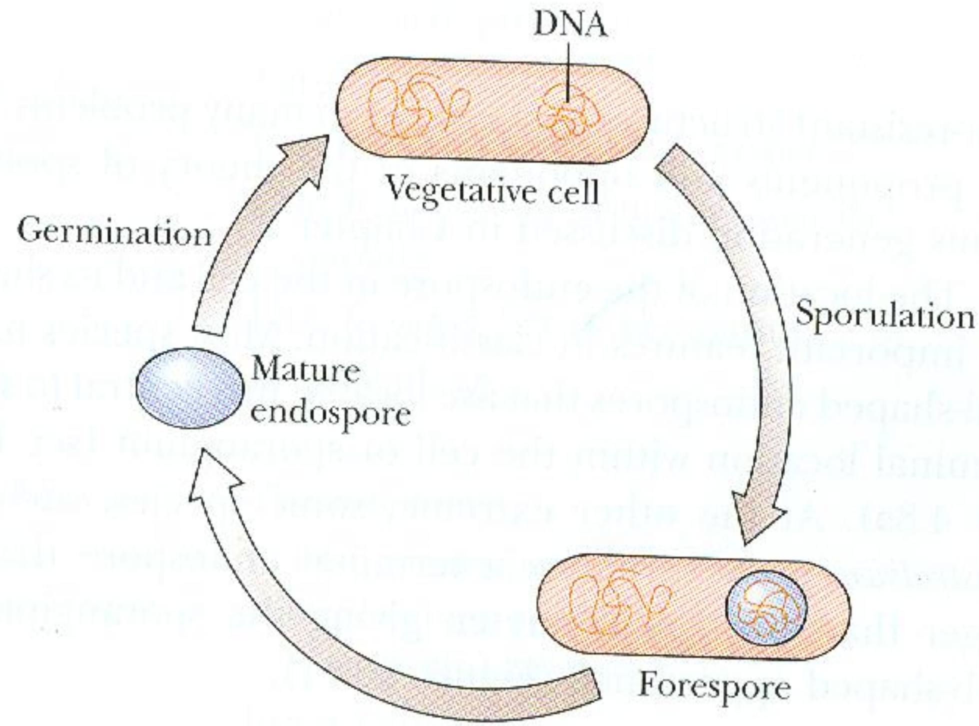


Figure **20.9** Diagram of the life cycle of an endospore-forming bacterium. The vegetative cell forms an endospore when nutrients become depleted in the process of sporulation. Subsequently, when conditions for growth are favorable the endospore can germinate to form a vegetative cell again.

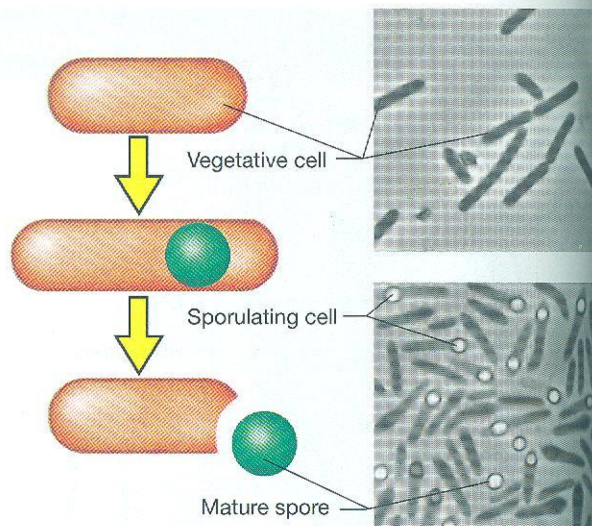
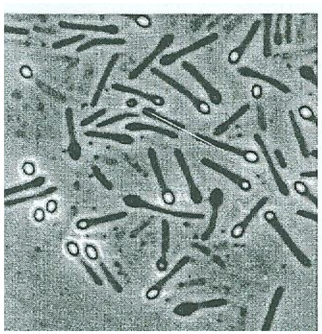
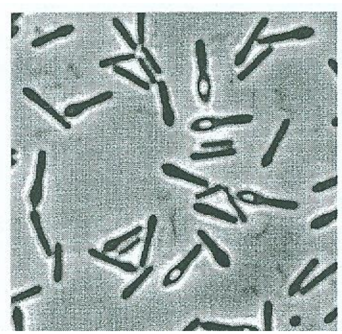
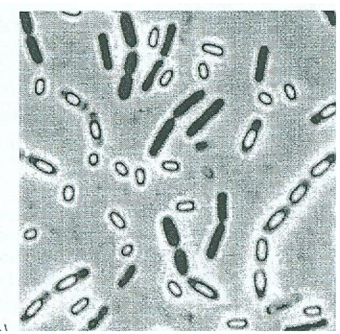


FIGURE 3.66 Formation of the endospore. Phase contrast photomicrographs are of cells of *Clostridium pascui*.



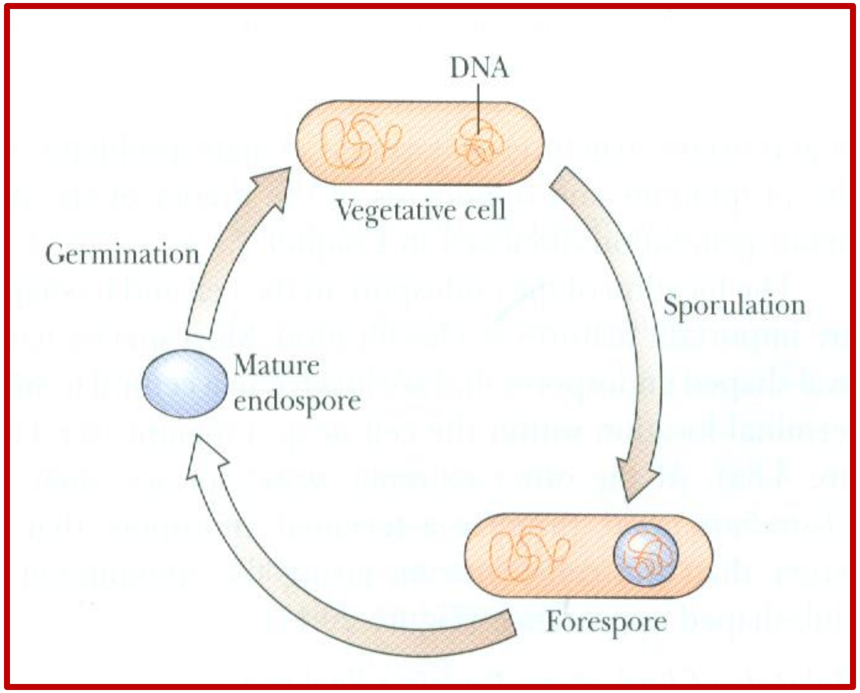




INDUTORES DA ESPORULAÇÃO

- 1.LIMITAÇÃO DE NUTRIENTES:
- PRINCIPALMENTE A FONTE DE NITROGÊNIO.
- O TEMPO DE ESPORULAÇÃO É DE DEZ HORAS.

CELULA VEGETATIVA, ESPORULAÇÃO E ESPORO



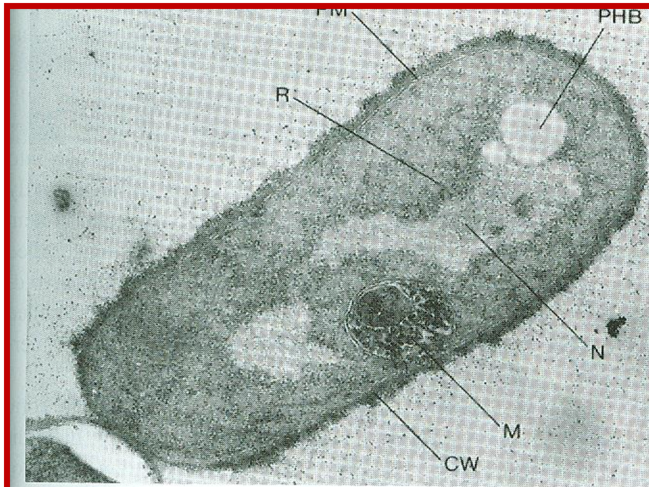
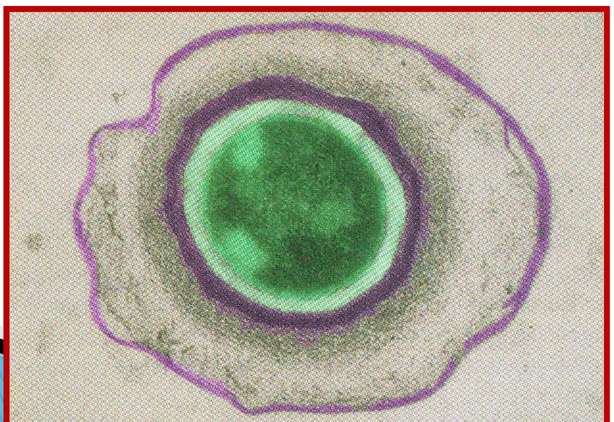
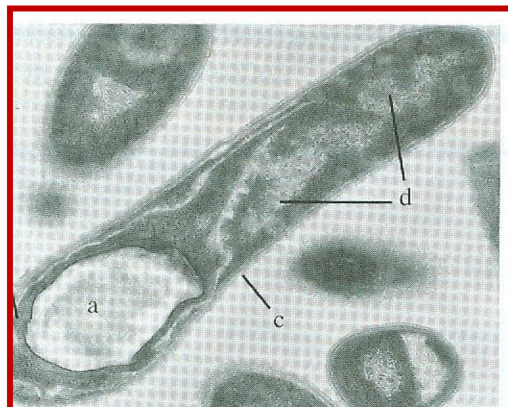


Figure 3.11 The Structure of a Typical Gram-Positive Cell. Electron micrograph of *Bacillus megaterium* (×30,500). Note the thick cell wall, CW; "mesosome," M; nucleoid, N; poly-β-hydroxybutyrate inclusion body, PHB; plasma membrane, PM; and ribosomes, R.





POLIMERO D0 ÁCIDO DIAMINOPIMÉLICO – DAP

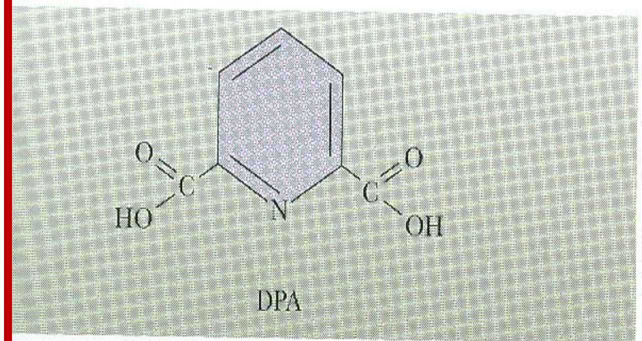
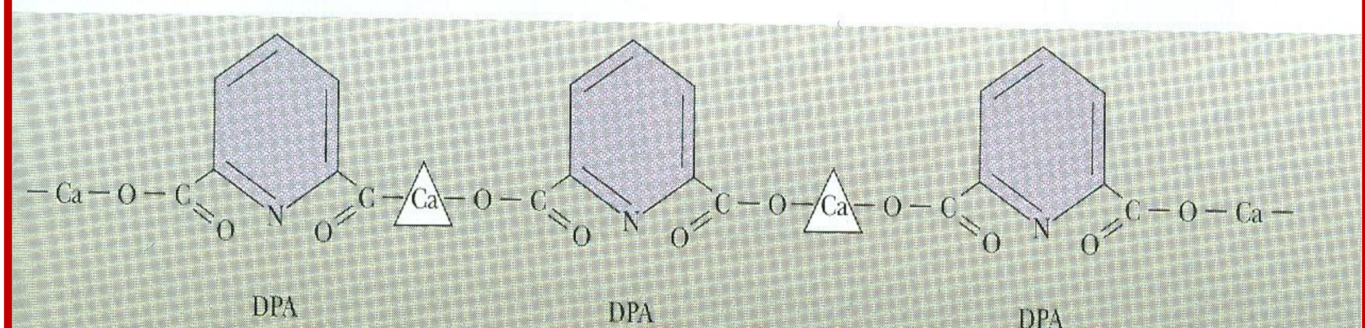


Figure **20.8** **(a)** Chemical formula of dipicolinic acid (DPA). **(b)** Pr
structure of the calcium-dipicolinic acid chelate.



FASES DA ESPORULAÇÃO B. *substilis*

DESCRIÇÃO DOS OITO ESTÁGIOS:

0. FORMAÇÃO DO FILAMENTOS AXIAL:

O cromossomo passa de estrutura compacta e superenrolada para um filamento.

1. FORMAÇÃO DO SEPTO: Invaginação da membrana citoplasmática.

Replicação do DNA e síntese de várias enzimas

2. ENGOLFAMENTO:

A EXTREMIDADE DO SEPTO MIGRA NA DIREÇÃO DO PRÉ-ENDOSPORO, ENVOLVENDO-O POR UMA CAMADA DUPLA DE MEMBRANA E LIVRE DO CITOPLASMA DA CELULA MÃE → PROTOPLASTO

FASES DA ESPORULAÇÃO

3. FORMAÇÃO DA PEPTIDEOGLICANA

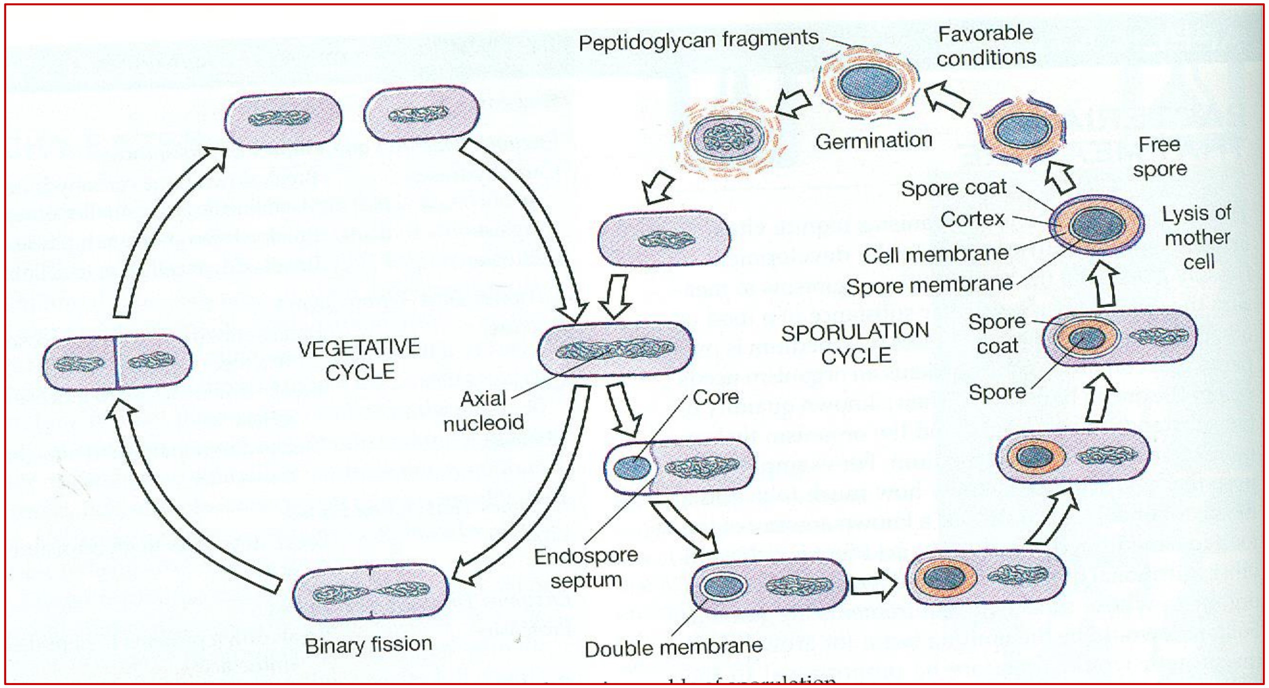
É DEPOSITADO ENTRE AS MEMBRANAS QUE ENVOLVEM O PRÉ-ENDOSPORO. A PAREDE TEM DUAS CAMADAS; A INTERNA QUE IRÁ TRANSFORMAR-SE NA PEPTIDEOGLICANA APÓS GERMINAÇÃO E O CÓRTEX QUE MANTEM O ESTADO DESIDRATADO DO ENDOSPORO. O CÓRTEX É FORMADO DE VARIAS CAMADAS DE PEPTIDEOGLICANA.

4. FORMAÇÃO DA CAPA

PROTEINAS COM AMINOÁCIDO CISTEINA OU GLICOPROTEÍNAS.

5. LIBERAÇÃO DO ENDOSPORO MADURO

A CELULA-MÃE LISA E LIBERA O ENDOSPORO MADURO.


Peptidoglycan fragments
Favorable conditions
Germination
Free spore
Spore coat
Cortex
Cell membrane
Spore membrane
Lysis of mother cell
SPORULATION CYCLE
Spore coat
Spore
VEGETATIVE CYCLE
Axial nucleoid
Core
Endospore septum
Double membrane
Binary fission

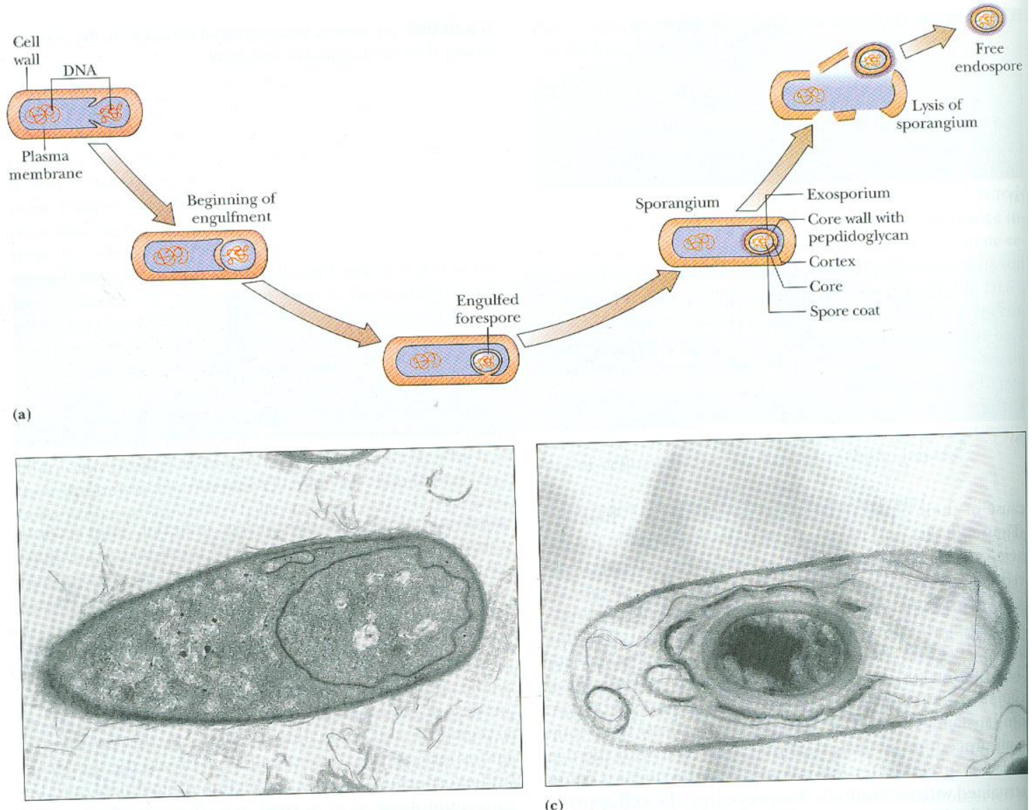
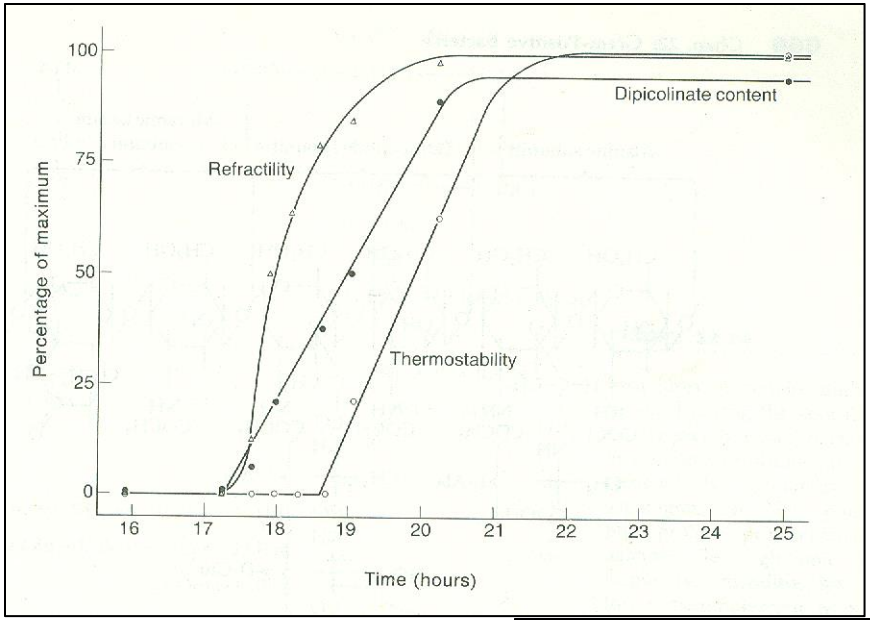


Figure **20.10** **(a)** Illustrations of the stages of sporulation as determined from electron micrographs. Thin sections through an endospore forming bacterium at **(b)** early stage of spore formation and **(c)** the mature spore. (Courtesy of S. Pankratz)



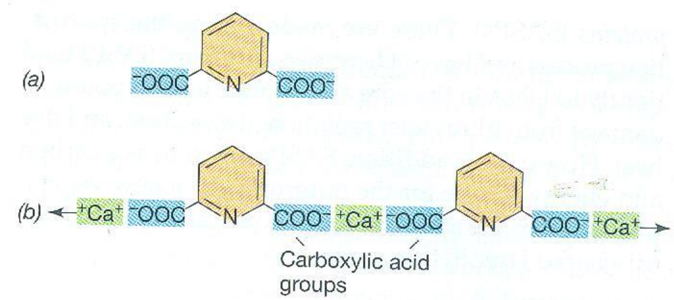


FIGURE 3.65 Dipicolinic acid (DPA). (a) Structure of DPA. (b) How Ca^{2+} cross-links DPA molecules to form a complex.

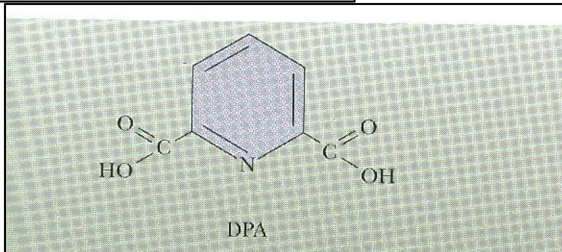
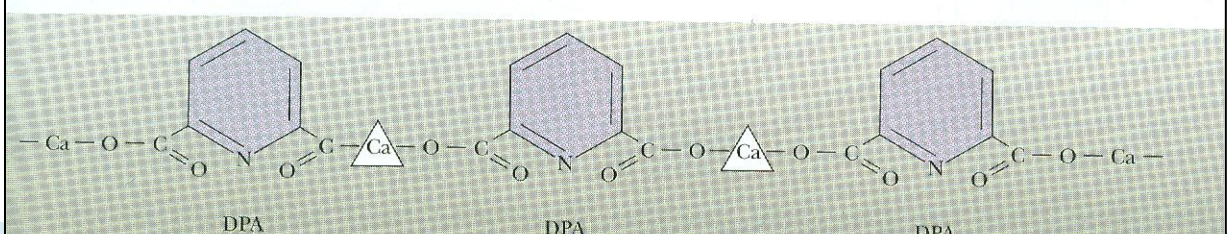


***Figure* 20.8** **(a)** Chemical formula of dipicolinic acid (DPA). **(b)** Pr
structure of the calcium-dipicolinic acid chelate.



FORMAÇÃO DO ENDOSPORO

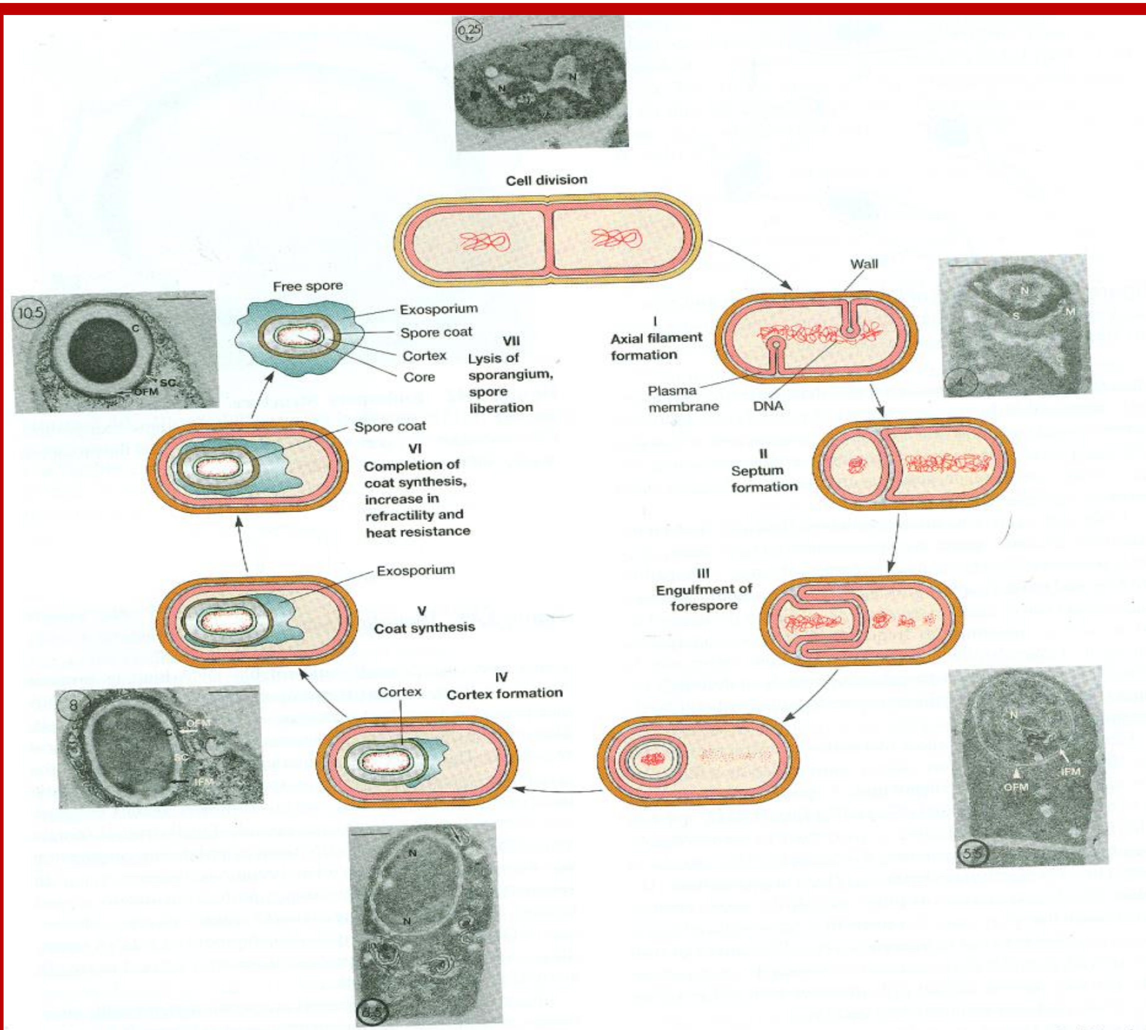


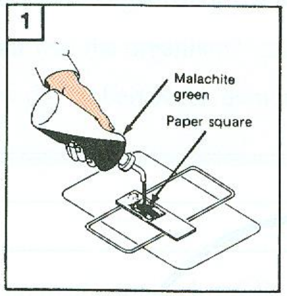
Figure 3.44 Endospore Formation: Life cycle of *Bacillus megaterium*. The stages are indicated by Roman numerals. The circl numbers in the photographs refer to the hours from the end of the logarithmic phase of growth: 0.25 h—a typical vegetative cell; 4 h—sta II cell, septation; 5.5 h—stage III cell, engulfment; 6.5 h—stage IV cell, cortex formation; 8 h—stage V cell, coat formation; 10.5 h—stage cell, mature spore in sporangium. Abbreviations used: C, cortex; IFM and OFM, inner and outer forespore membranes; M, mesosome; nucleoid; S, septum; SC, spore coats. Bars = 0.5 µm.

GENES ENVOLVIDOS NA ESPORULAÇÃO

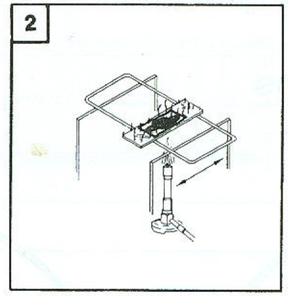
TABLE 19-2. A Partial List of Sporulation Genes and Their Function in *Bacillus subtilis*

Stage	Gene Designation	Function
I	*citC*	Isocitrate dehydrogenase
IIi	*spoOA*	Response regulator; phosphorylation initiates sporulation via phosphorelay system
	spoOK	Regulates phosphorelay system
	spoOH	Encodes σ^H
IIii	*spoIIG*	Pro σ^E and activating protease
	spoIIB + *spoVG*	Prespore engulfment; septum formation
IIiii	*spoIID*	Septal peptidoglycan hydrolysis
	spoIIA(P)	Processing of pro σ^E
III	*spoIIIA*	Prespore engulfment
	SpoIIIE	Controls σ^F expression
	spoIIiJ (kinA)	Histidine protein kinase; activates SpoOF
	spoIIID	Controls σ^E-dependent genes
	spoIIIG	Structural gene for σ^G
	spoIVB	Production of σ^K; signal peptide
	spoIVF (spoIIIF)	Processing of pro-σ^K
	spoVB	Cortex synthesis
	spoVD	Cortex synthesis
	spoVE	Cortex synthesis
IV	*cotD*	Coat synthesis
	cotT	Coat synthesis
	cotA	Coat synthesis
	cotB	Coat synthesis
	cotC	Coat synthesis
	gerE	Germination

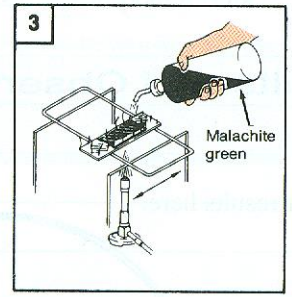
COLORAÇÃO DO ESPORO



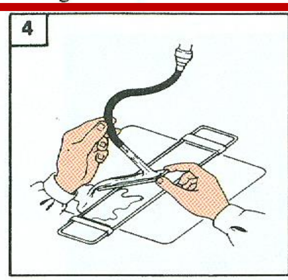
1. Place the slide on the staining rack. Cover the smear with a small paper towel square and then with malachite green. Allow the stain to stand for 30 to 60 sec. before heating.



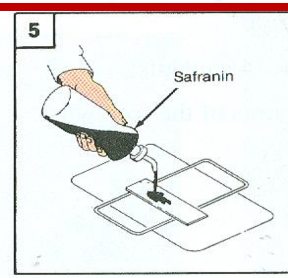
2. Heat the preparation gently by passing the Bunsen burner under the slide. Continue heating until you see a slight steaming when the flame is removed.



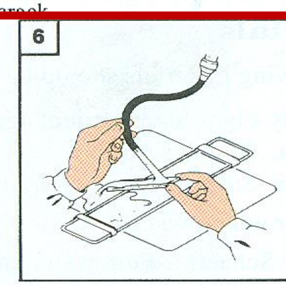
3. Maintain steaming for 5 min. Add more dye as needed to prevent the smear from drying out. Be careful not to overheat the slide. Overheated slides may crack.



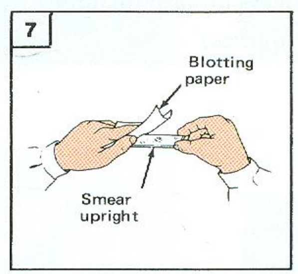
4. Allow the slide to cool, remove the paper towel square, and rinse the slide (front and back) in slowly running water.



5. Apply the counterstain, safranin, for 1 min.

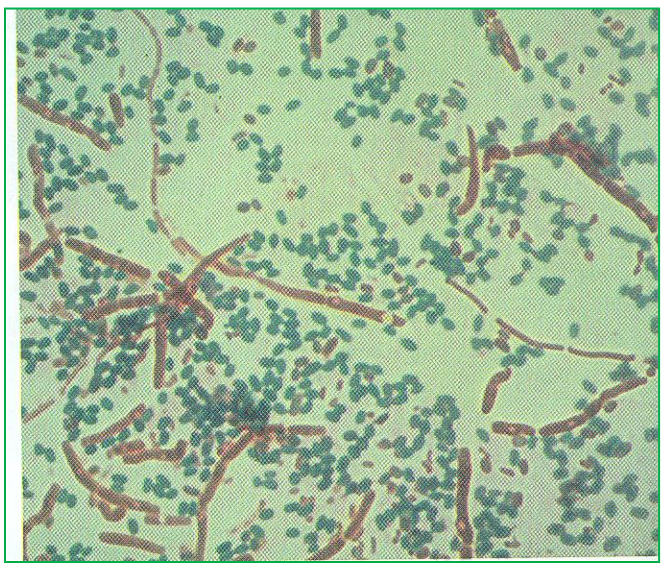


6 Rinse the smear with running water as in step 4.



7. Blot dry and observe as directed earlier.

ESPOROS APÓS COLORAÇÃO



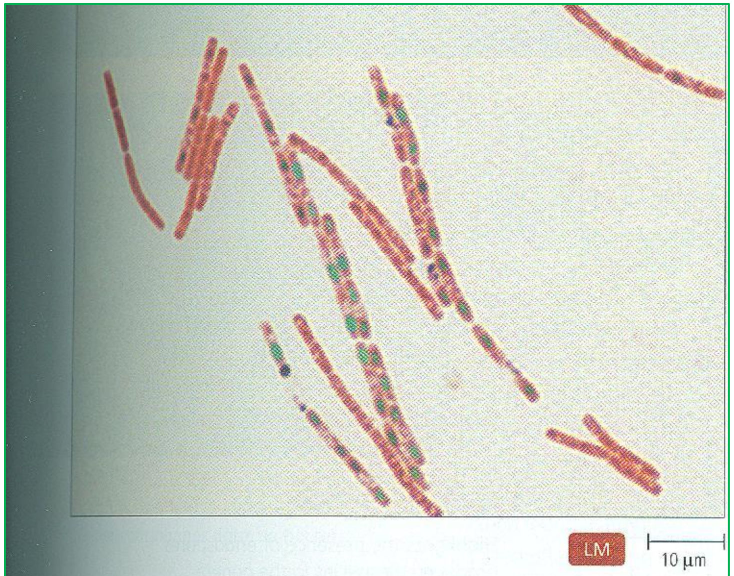
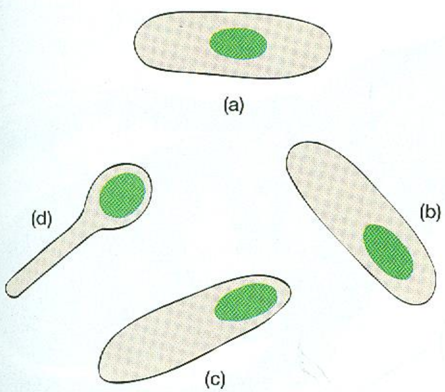
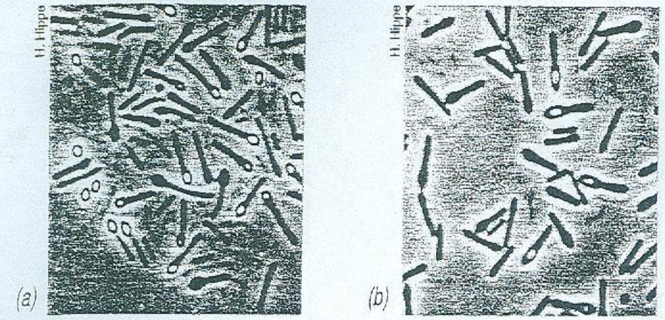


Figure 4.19

Schaeffer-Fulton endospore stain of *Bacillus anthracis*. The nearly impermeable spore wall retains the green dye during decolorization. Vegetative cells, which lack spores, pick up the counterstain and appear red. *Why don't the spores stain red as well?*



3.41 Examples of Endospore Location and Size.
tral spore. (**b**) Subterminal spore. (**c**) Terminal spor
inal spore with swollen sporangium.



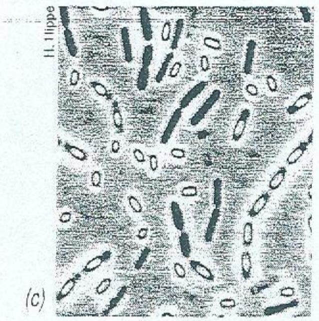


Figura 4.59 Endósporos bacterianos. Fotomicrografias de contraste de fase ilustrando vários tipos morfológicos e localizações intracelulares dos endósporos. (a) Terminais. (b) Subterminais. (c) Centrais.

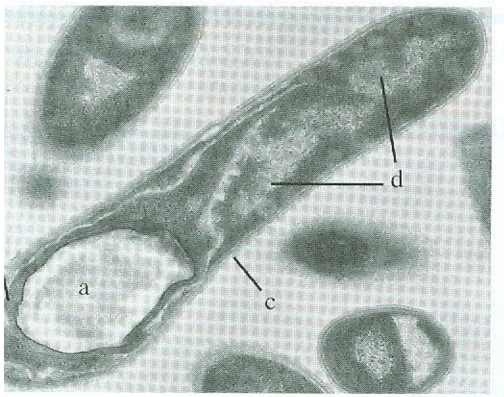
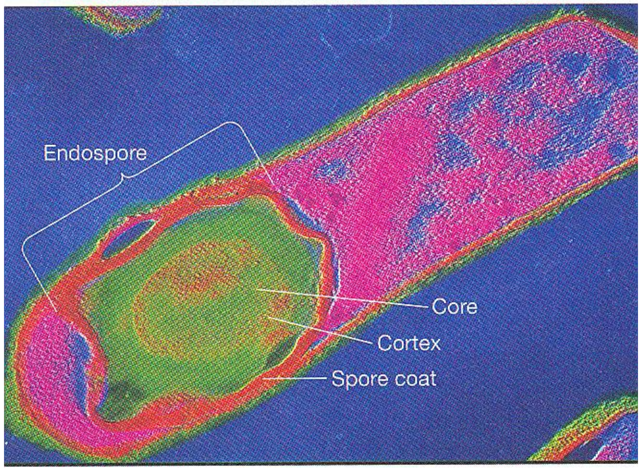


FIGURE 4.10 Color-enhanced electron micrograph of an endospore within a *Clostridium perfringens* cell.



MICROFOTOGRAFIA DE ENDOSPORO DE *Bacillus*

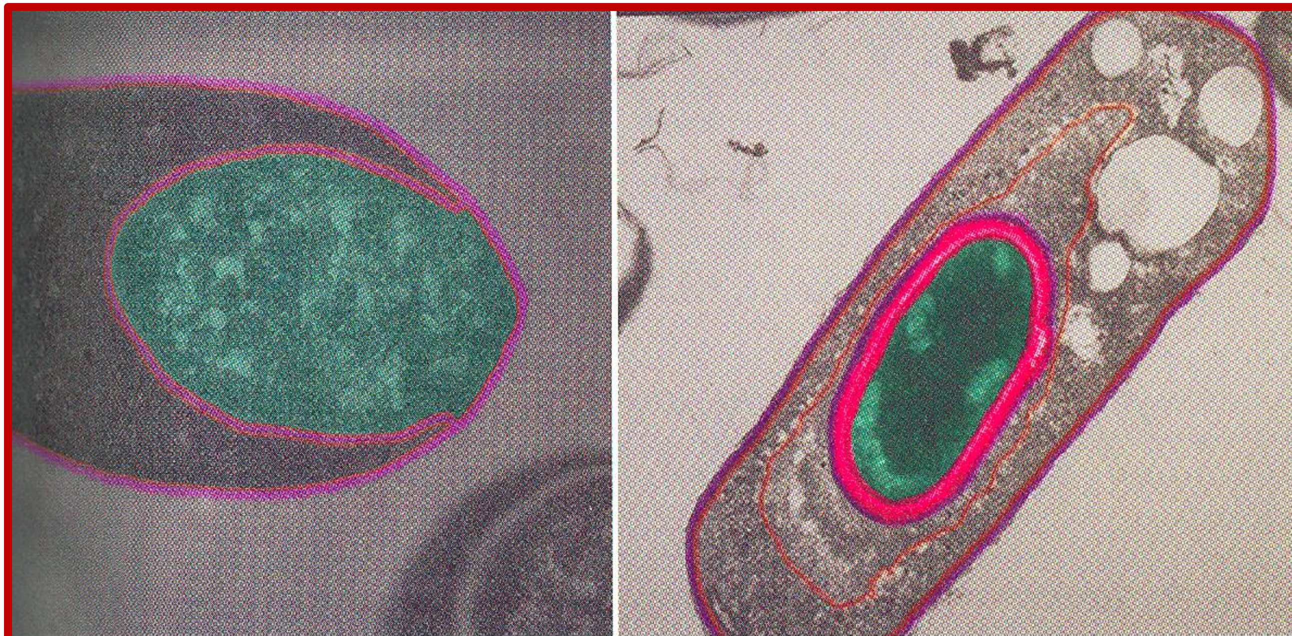


Fig. 9-13 Formation of Bacterial Endospore. **A,** Colorized micrograph of *Bacillus* during formation of an endospore. (40,600×.) **B,** Colorized micrograph of *Bacillus* after formation of an endospore. (32,400×.)

MICROFOTOGRAFIA DE ENDOSPORO DE *Clostridium perfringens*

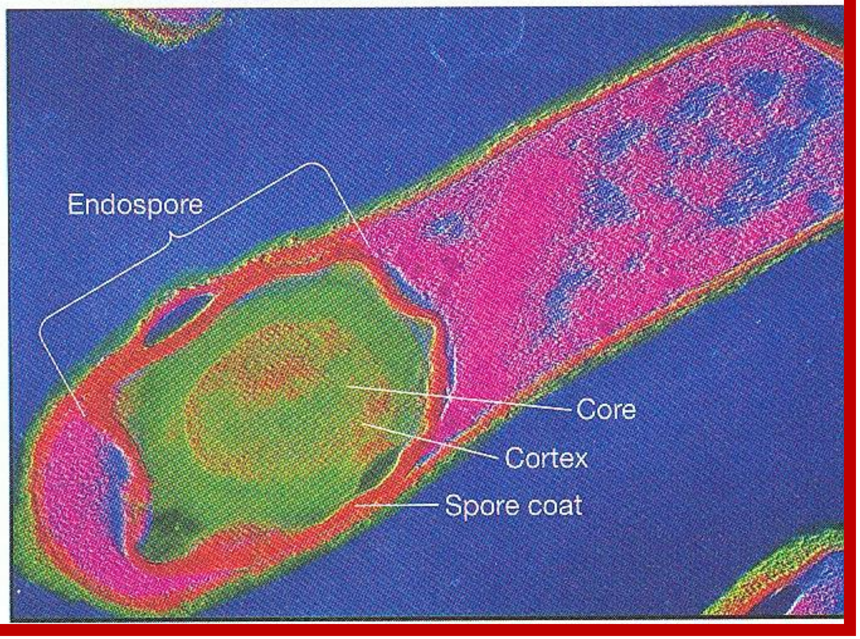


FIGURE 4.10 Color-enhanced electron micrograph of an endospore within a *Clostridium perfringens* cell.

MICROFOTOGRAFIA DE ENDOSPORO DE *Clostridium thuringiensis*

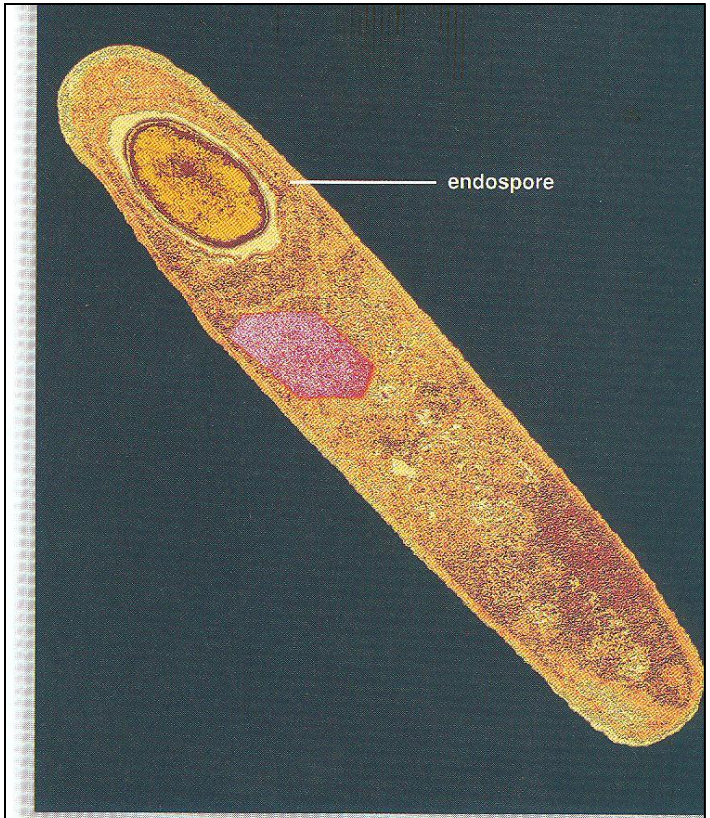
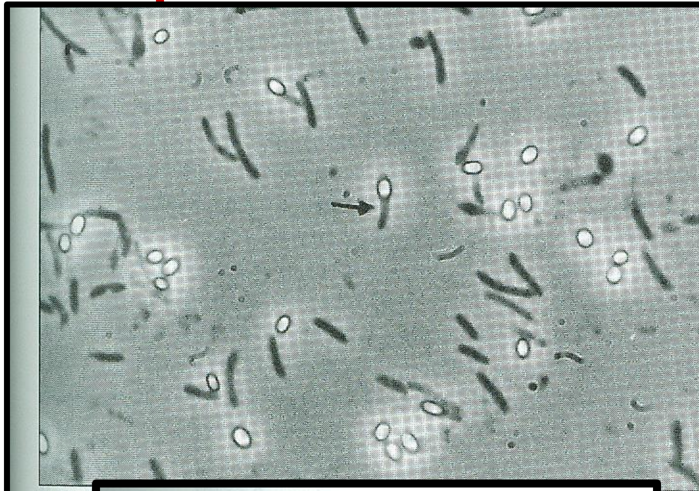
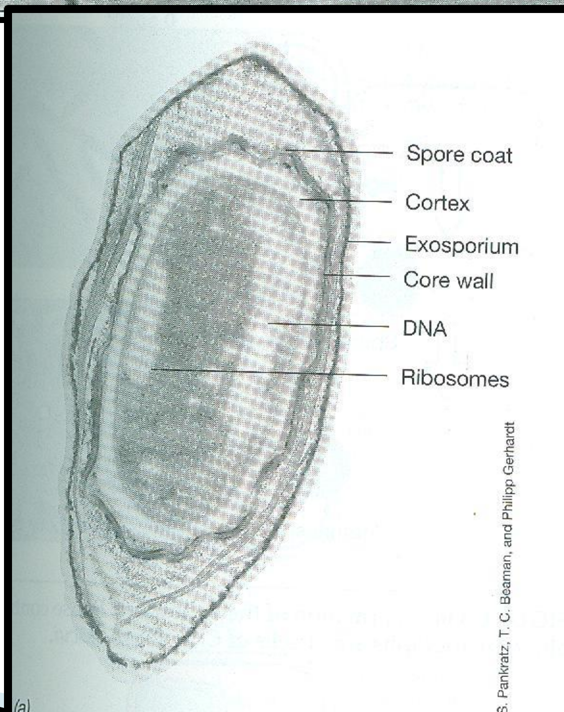


Figure 4.21 **Endospore inside *Bacillus thuringiensis*.** The genus *Bacillus* forms endospores. *B. thuringiensis* additionally forms crystalline bodies (pink) that are used as insecticides.

ENDOSPOROS





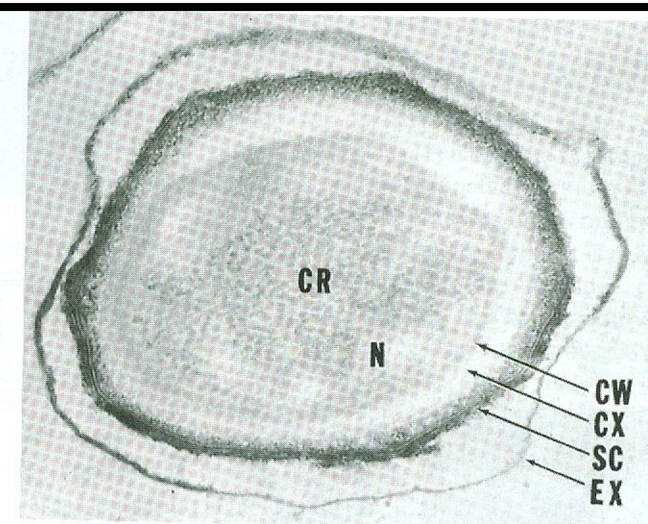
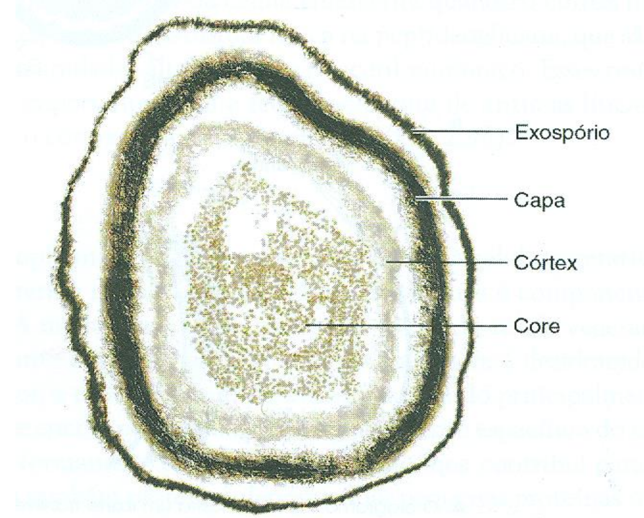
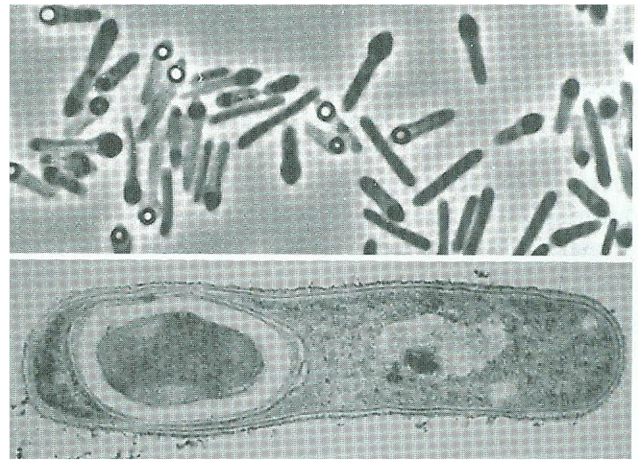
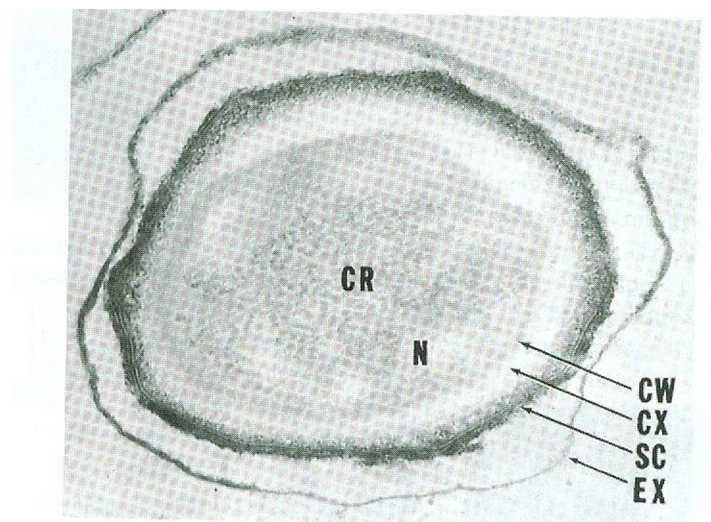


Figure 3.42 Endospore Structure. *Bacillus anthracis* endospore (×151,000). Note the following structures: exosporium, EX; spore coat, SC; cortex, CX; core wall, CW; and the protoplast or core with its nucleoid, N, and ribosomes, CR.







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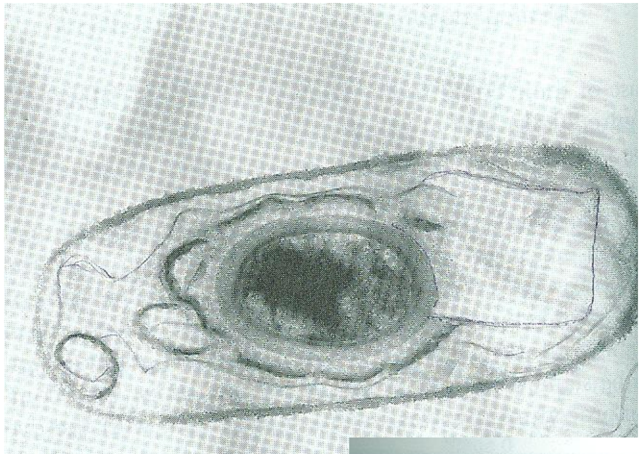
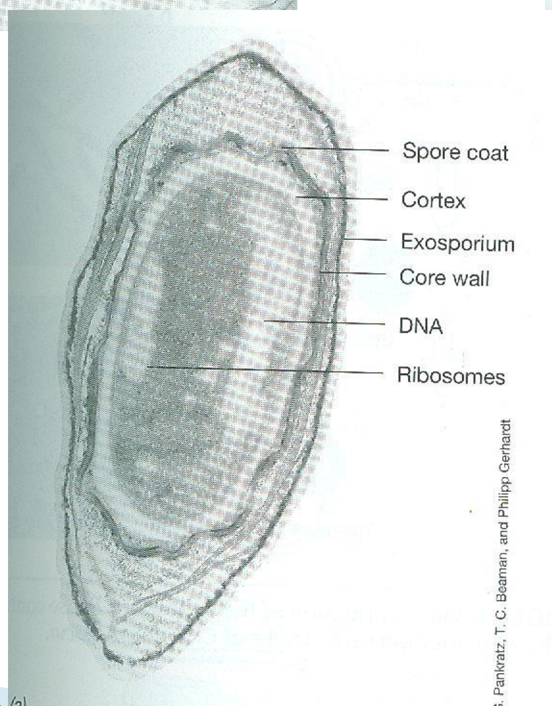




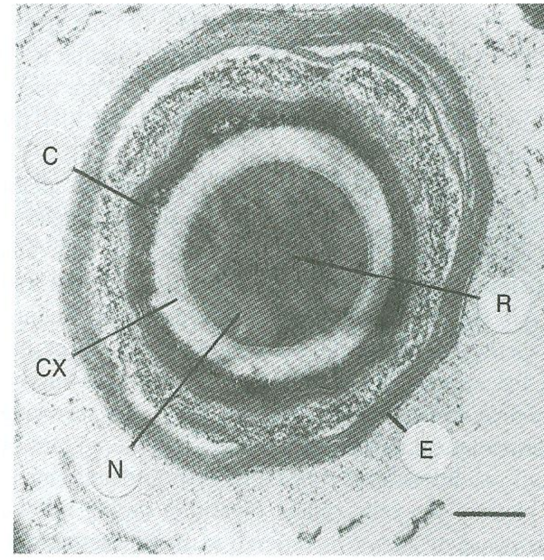
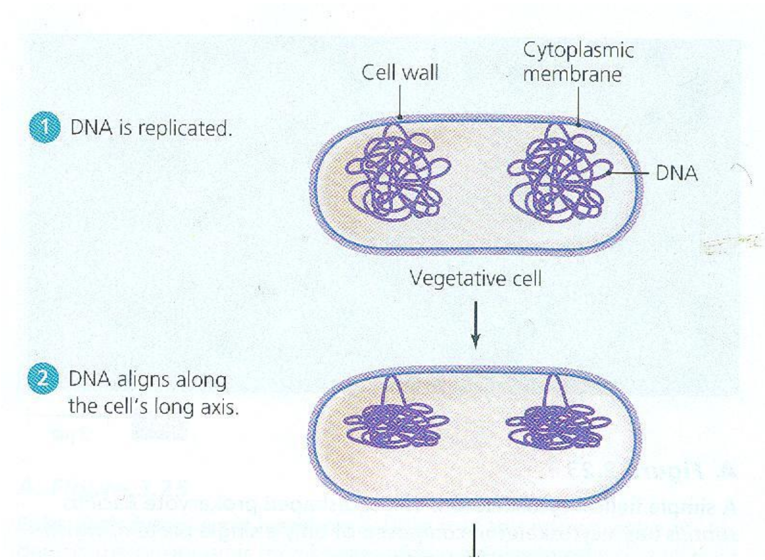
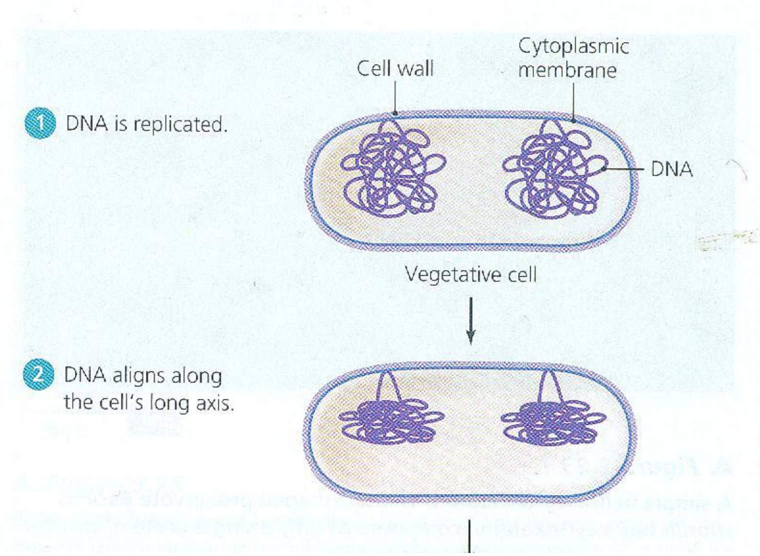


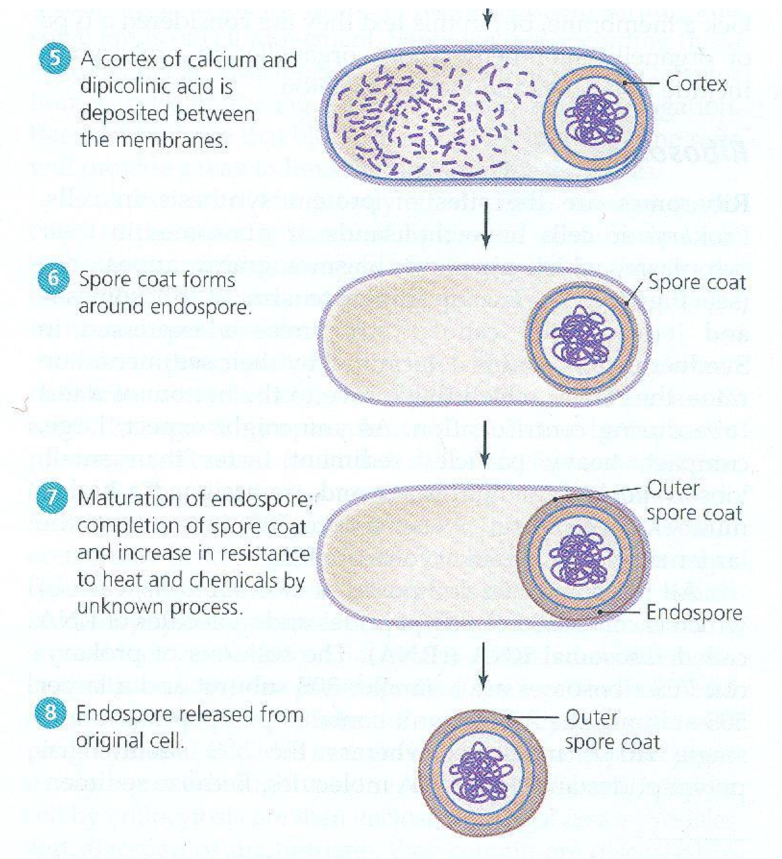
Fig. 1-15. Mature spore of *Clostridium botulinum*. Shown is a well-defined, multilayered exosporium (E), an electron-dense outer coat layer, a thick inner coat (C) and a less dense cortex (CX). The darkly stained ribosomes (R) and nucleoid areas (N) are clearly differentiated in the spore interior. Bar equals 0.2 µm. (*Source*: From Stevenson, K. E., R. H. Vaughn, and E. V. Crisan, 1972. *J. Bacteriol.* **109:**1295.)

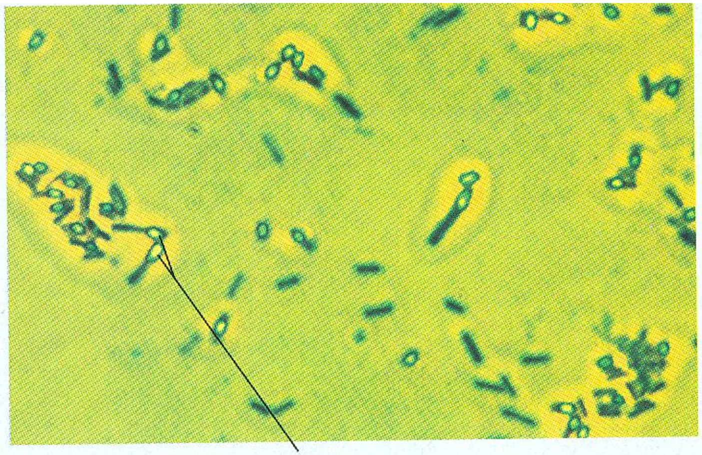


Cell wall
Cytoplasmic
membrane
1 DNA is replicated.
DNA
Vegetative cell
2 DNA aligns along
the cell's long axis.

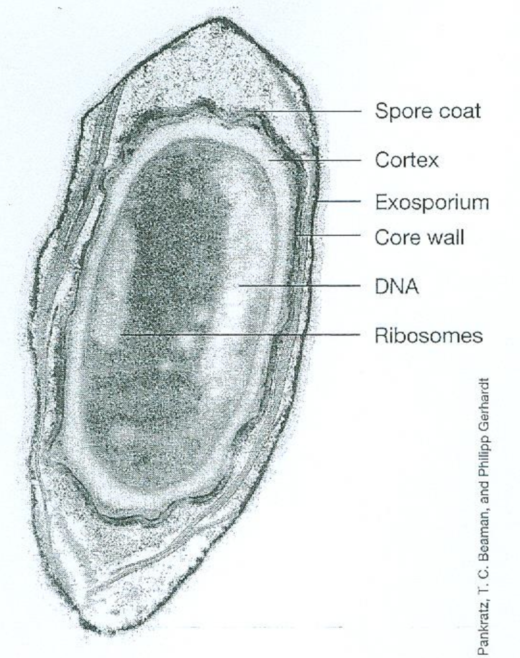


1 DNA is replicated.
Cell wall
Cytoplasmic membrane
DNA
Vegetative cell
2 DNA aligns along the cell's long axis.


5 A cortex of calcium and dipicolinic acid is deposited between the membranes.
Cortex
6 Spore coat forms around endospore.
Spore coat
7 Maturation of endospore; completion of spore coat and increase in resistance to heat and chemicals by unknown process.
Outer spore coat
Endospore
8 Endospore released from original cell.
Outer spore coat


Bacterial spores

(a)


Spore coat
Cortex
Exosporium
Core wall
DNA
Ribosomes
Pankratz, T. C. Beaman, and Philipp Gerhardt

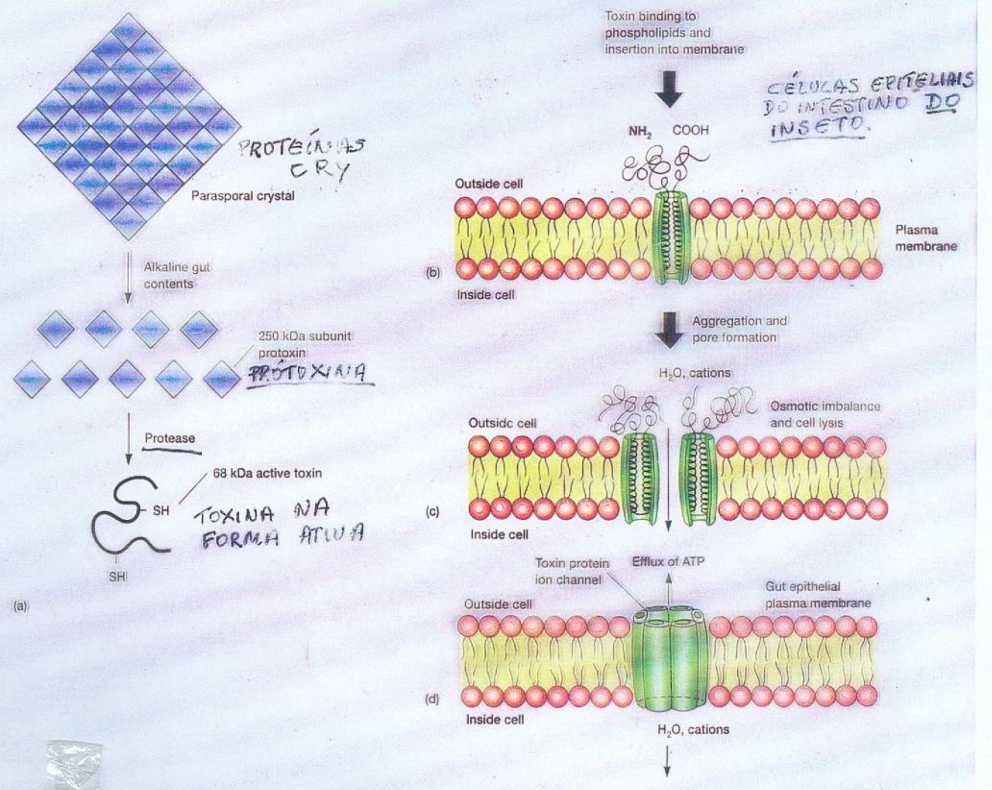


Figure 42.12 **The Mode of Action of the *Bacillus thuringiensis* Toxin.** **(a)** Release of the protoxin from the parasporal body and modification by proteases in the hindgut. **(b)** Insertion of the 68 kDa active toxin molecules into the membrane. **(c)** Aggregation and pore formation, showing a cross section of the pore. **(d)** Final creation of the hexagonal pore which causes an influx of water and cations as well as a loss of ATP, resulting in cell imbalance and lysis.

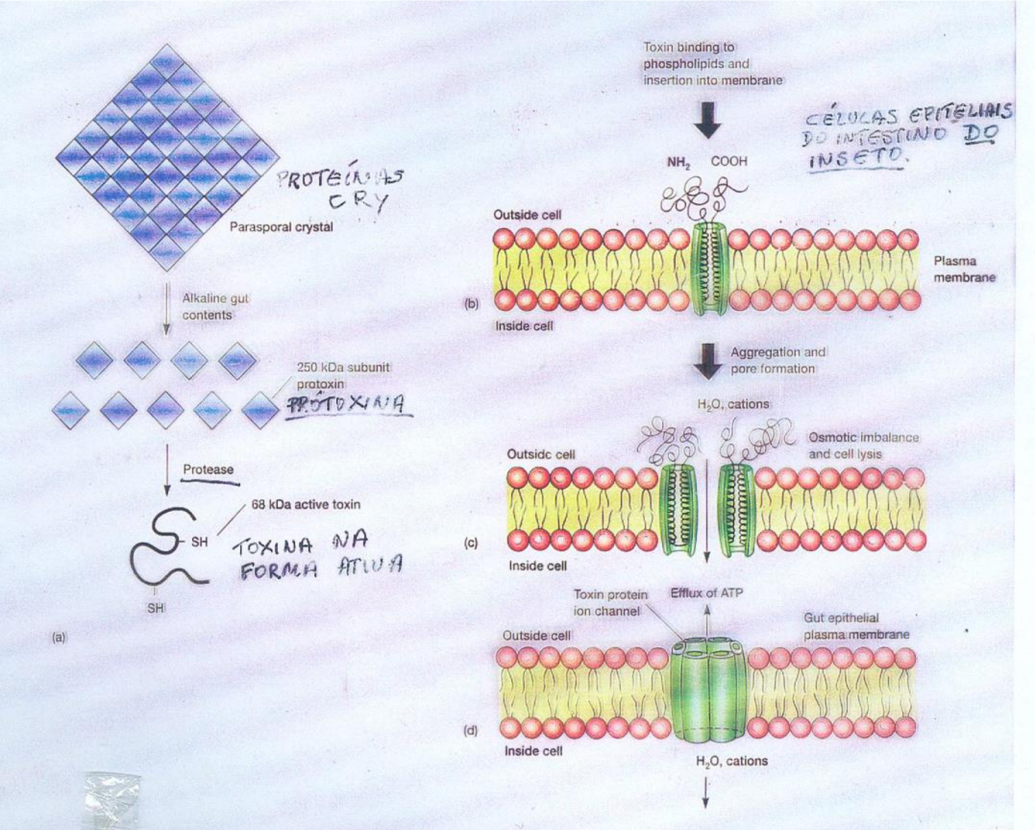


Figure 42.12 The Mode of Action of the *Bacillus thuringiensis* Toxin. **(a)** Release of the protoxin from the parasporal body and modification by proteases in the hindgut. **(b)** Insertion of the 68 kDa active toxin molecules into the membrane. **(c)** Aggregation and pore formation, showing a cross section of the pore. **(d)** Final creation of the hexagonal pore which causes an influx of water and cations as well as a loss of ATP, resulting in cell imbalance and lysis.

TABLE 3.2 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
Heat resistance	Low	High
Radiation 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)

TABLE 4.1 General Stages in Endospore Formation

Stage	State of Cell	Process/Event
1	Vegetative cell	Cell in early stage of binary fission doubles chromosome.
2	Vegetative cell becomes **sporangium** in preparation for sporulation.	One chromosome and a small bit of cytoplasm are walled off as a protoplast at one end of the cell. This core contains the minimum structures and chemicals necessary for guiding life processes. During this time, the sporangium remains active in synthesizing compounds required for spore formation.
3	Sporangium	The protoplast is engulfed by the sporangium to continue the formation of various protective layers around it.
4	Sporangium with prospore	Special peptidoglycan is laid down to form a cortex around the spore protoplast, now called the prospore; calcium and dipicolinic acid are deposited; core becomes dehydrated and metabolically inactive.
5	Sporangium with prospore	Three heavy and impervious protein spore coats are added.
6	Mature endospore	Endospore becomes thicker, and heat resistance is complete; sporangium is no longer functional and begins to deteriorate.
7	Free spore	Complete lysis of sporangium frees spore; it can remain dormant yet viable for thousands of years.
8	Germination	Addition of nutrients and water reverses the dormancy. The spore then swells and liberates a young vegetative cell.
9	Vegetative cell	Restored vegetative cell


Fluorescent stai
of *Bacillus subtili*


TEM of cross section of free endospore