

SPORES

(endospores)

the spore is formed inside the parent vegetative cell – hence the name „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, minimum concentrations of essential proteins and ribosomes, and a high concentration of calcium bound to dipicolinic acid.

Members of several bacterial genera are capable of forming endospores:

- *Bacillus anthracis*
- *Clostridium tetani*
- *Clostridium botulinum*
- *Clostridium perfringens*
- and other, but never Gram-negative microbes

- Spore formation is a means by which some bacteria are able to survive extremely harsh environmental conditions.
- The genetic material of the bacterial cells is concentrated and then surrounded by a protective coat, rendering the cell impervious to desiccation, heat and many chemical agents.

- The bacteria in the stage of spore is metabolically inert and can remain stable for months to years. When exposed to favorable conditions, germination can occur, with the production of single cell that subsequently can undergo normal replication.
- It should be obvious that the complete eradication of disease caused by spore-forming microorganisms is difficult or impossible.

- The two major groups of bacteria that form spores are the aerobic genus *Bacillus* (e.g. disease anthrax) and the anaerobic genus *Clostridium* (e.g. disease tetanus, botulinism).

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 series of genes whose products determine the formation and final composition of the spore are activated, while another series of genes involved in vegetative cell function are inactivated.

Sporulation

- Morphologically, sporulation begins with the isolation of a nucleoid 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.

Properties of endospores

Core

- The core is the spore protoplast.
- It contains a complete nucleoid (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 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.

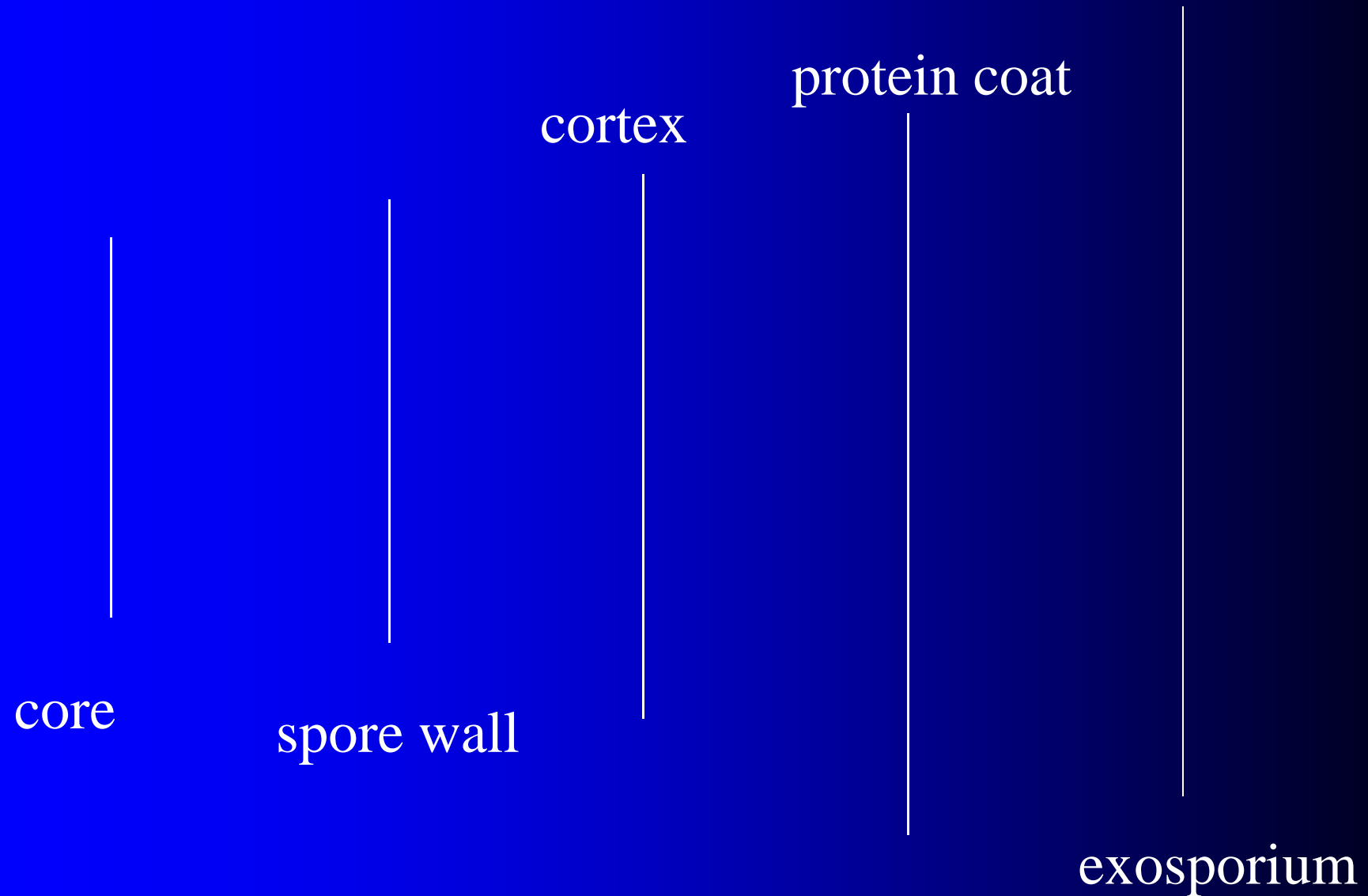
Coat

- 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.

Spore structure - schematic



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 compounds 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.
- Outgrowth requires a supply of all nutrients essential 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 heating 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.