۞۞۞ Spore-Forming Gram-Positive Bacilli ۞۞۞

- ***Bacillus Species*** **(ex:** *B.anthracis ; B.cereus*)
- ***Clostridium Species*** **(ex:** *C.perfringens ; C.tetani; C. difficile*)

Sporulation:

The sporulation process begins when nutritional conditions become unfavorable, near depletion of the nitrogen or carbon source (or both) Many environmental bacteria are able to produce stable dormant, or resting, forms as a branch of their life cycle to enhance their survival under adverse conditions. Such dormant forms are called endospores.

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.

Morphologically, sporulation begins with the formation of an axial filament . The process continues with an in folding 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.

Germination:

The germination process occurs in three stages: activation, initiation, and outgrowth:

1-Activation

Most endospores cannot germinate immediately after they have formed. But they can germinate after they have rested for several days or are first activated, in a nutritionally rich medium, 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.

2-Initiation

Once activated, a spore will initiate germination if the environmental conditions are favorable. Different species have evolved receptors that recognize different effectors as signaling a rich medium: Thus, initiation is triggered by L-alanine in one species and by adenosine in another. 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.

3- 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. Outgrowth requires a supply of all nutrients essential for cell growth.

Bacillus Species

The genus *Bacillus* includes large, aerobic, gram-positive rods occurring in chains. Most members of this genus are saprophytic organisms prevalent in soil, water, and air and on vegetation, such as *Bacillus cereus* and *Bacillus subtilis* . Some are insect pathogens, such as *B.thuringiensis* . This organism is also capable of causing disease in humans. *B cereus* can grow in foods and cause **food poisoning** by producing either an enterotoxin (diarrhea) or an emetic toxin (vomiting). Both *B cereus* and B *thuringiensis* may occasionally produce disease in immune compromised humans (eg, meningitis, endocarditis, endophthalmitis, conjunctivitis, or acute gastroenteritis). *B anthracis* , which causes **anthrax, is** the principal pathogen of the genus.

Morphology and identification

A. Typical Organisms

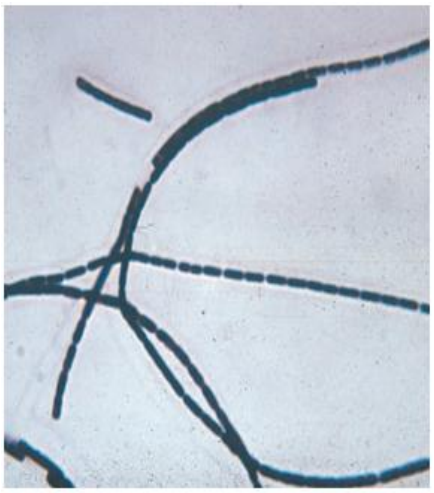
The typical cells, measuring 1 × 3–4 μm, have square ends and are arranged in long chains; spores are located in the center of the bacilli.

B. Culture

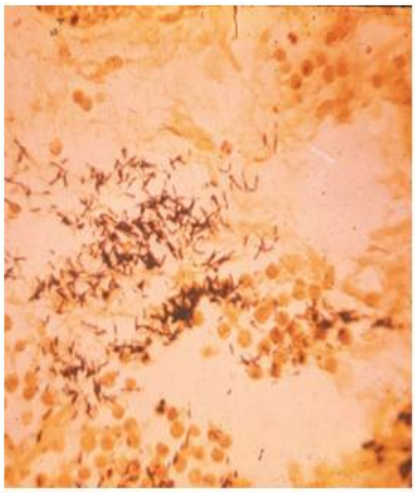
Colonies of *B anthracis* are round and have a *"cut glass"* appearance in transmitted light. Hemolysis is uncommon with *B anthracis* but common with *B cereus* (type β) and the saprophytic bacilli. Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree.

C. Growth Characteristics

The saprophytic bacilli use simple sources of nitrogen and carbon for energy and growth. The spores are resistant to environmental changes, withstand dry, heat and certain chemical disinfectants for moderate periods, and persist for years in dry earth. Animal products contaminated with anthrax spores can be sterilized by autoclaving.



A: *Bacillus anthracis in broth culture*



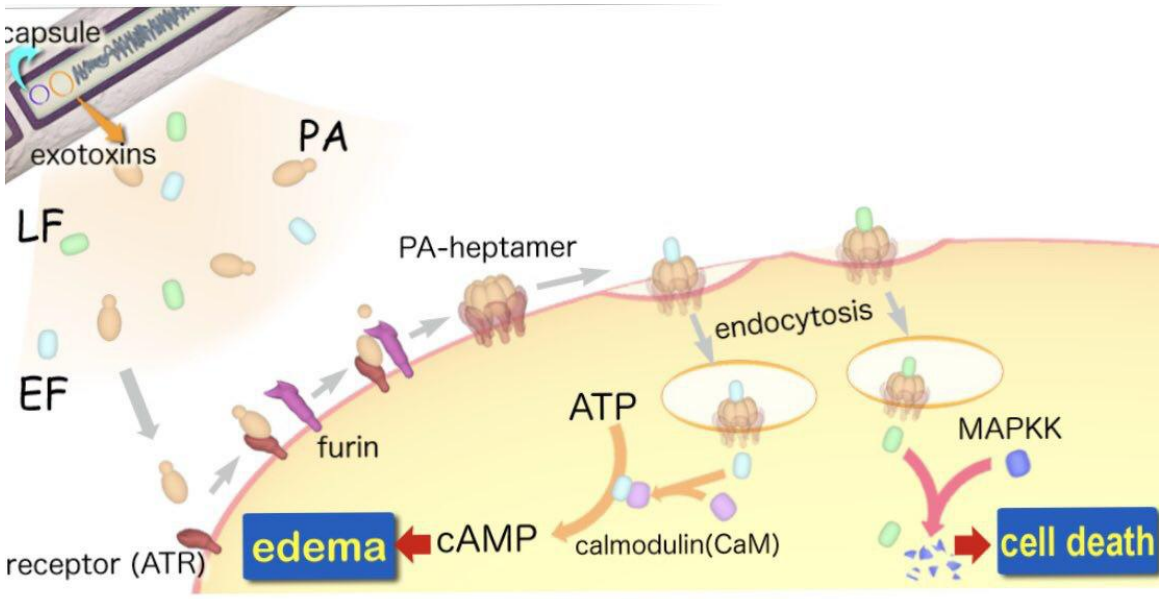
B: In tissue

Pathogenesis:

Anthrax is primarily a disease of herbivores goats, sheep, cattle, horses. Humans become infected incidentally by contact with infected animals or their products. the infection is usually acquired by the entry of spores through injured skin **(cutaneous anthrax)** or rarely the mucous membranes **(gastrointestinal anthrax)** or by inhalation of spores into the lung **(inhalation anthrax).** The spores germinate in the tissue at the site of entry, and growth of the vegetative organisms results in formation

of a gelatinous edema and congestion. Bacilli spread via lymphatic's to the bloodstream, and they multiply freely in the blood and tissues shortly before and after the animal's death.

Anthrax toxins are made up of three proteins, **protective antigen (PA), edema factor (EF), and lethal factor (LF).** PA binds to specific cell receptors, and after proteolytic activation, it forms a membrane channel that mediates entry of EF and LF into the cell. EF is an adenylate cyclase; with PA, it forms a toxin known as **edema toxin**. LF plus PA form **lethal toxin**, which is a major virulence factor and cause of death in infected animals and humans.



Mechanism of anthrax toxin action

Diagnostic Laboratory Tests

A- Specimens to be examined are:

1- fluid or pus from a local lesion, Blood, Pleural fluid ,Cerebrospinal fluid In inhalational anthrax associated with sepsis
2- Stool or other intestinal contents in the case of gastrointestinal anthrax.

B- Staining smears often show chains of large gram-positive rods.

C- Culture :

-- Demonstration of capsule requires growth on bicarbonate-containing medium in 5–7% carbon dioxide.

-- anthrax bacilli are always non-motile, but related organisms (eg, *B cereus)* exhibit motility by *"swarming.*

Treatment

Many antibiotics are effective against anthrax in humans, but treatment must be started early. **Ciprofloxacin is recommended for treatment; penicillin G, along with gentamicin or streptomycin**, has previously been used to treat anthrax. In the setting of potential exposure to *B anthracis* as an agent of biologic warfare, prophylaxis with ciprofloxacin or doxycycline should be continued for 4 weeks while three doses of vaccine are being given or for 8 weeks if no vaccine is administered. Some other gram-positive bacilli, such as *B cereus, are* resistant to penicillin by virtue of β-lactamase production. Doxycycline, erythromycin, and ciprofloxacin may be effective alternatives to penicillin.

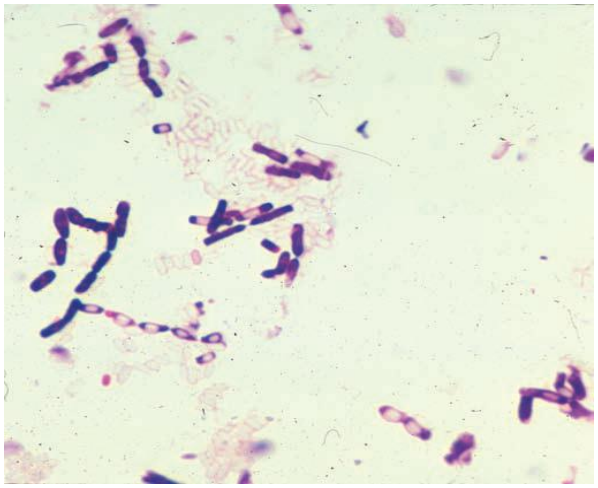
Clostridium Species

The clostridia are large **anaerobic**, gram-positive, motile rods. Many decompose proteins or form toxins, and some do both. Their natural habitat is the soil or the intestinal tract of animals and humans, where they live as saprophytes. Among the pathogens are the organisms causing **botulism, tetanus, gas gangrene, and pseudomembranous colitis.**

Morphology and Identification

A. Typical Organisms

Spores of clostridia are usually wider than the diameter of the rods in which they are formed. In the various species, the spore is placed **centrally**, **subterminally**, or **terminally**. Most species of clostridia are motile and possess peritrichous flagella. *Clostridium* Gram stain. Individual gram-positive bacilli are present. Many are in chains. Some of the bacilli have spores, which are the unstained or clear ovoid shapes.



Gram stain of ***Clostridium***

B. Culture

Clostridia are anaerobes and grow under anaerobic conditions; a few species are aero-tolerant and also grow in ambient air. In general, the clostridia grow well on the blood-enriched media or other media used to grow anaerobes.

C. Colony Forms

Some clostridia produce large raised colonies (eg, *C.perfringens);* others produce smaller colonies (eg, *C.tetani). Some clostridia* form colonies that spread on the agar surface. Many clostridia produce a zone of β-hemolysis on blood agar. *C perfringens* characteristically produces a double zone of β-hemolysis around colonies.

1-Clostridium botulinum:

Cl.botulinum, which causes **botulism, is worldwide in distribution;** it is found in soil and occasionally in animal feces. Types of *C.botulinum* are distinguished by the antigenic type of toxin they produce. Spores of the organism are highly resistant to heat, withstanding 100°C for several hours. Heat resistance is diminished at acid pH or high salt concentration.

- **Toxin**

During the growth of *C.botulinum* and during autolysis of the bacteria, toxin is liberated into the environment. **Seven antigenic varieties of toxin (A–G) are known. Types A, B, E, and F are the principal causes of human illness.** The toxin is a 150,000-MW (molecular weight) protein that is cleaved into 100,000- MW and 50,000-MW proteins linked by a disulfide bond. **B**otulinum toxin is absorbed from the gut and binds to receptors of presynaptic membranes of motor neurons of the peripheral nervous system and cranial nerves. inhibits the release of acetylcholine at the synapse, resulting in lack of muscle contraction and **paralysis**.

In infant botulism, honey is the most frequent vehicle of infection. The infant ingests the spores and the spores germinate within the intestinal tract. The vegetative cells produce toxin as they multiply; the neurotoxin then gets absorbed into the bloodstream. The toxin acts by blocking release of acetylcholine at synapses and neuromuscular junctions. The result is flaccid paralysis.

- **Clinical Findings**

Symptoms begin 18–24 hours after ingestion of the toxic food, with visual disturbances (incoordination of eye muscles, double vision), inability to swallow, and speech difficulty and death occurs from respiratory paralysis or cardiac arrest. Gastrointestinal symptoms are not regularly prominent. There is no fever. The patient remains fully conscious until shortly before death. The mortality rate is high. Patients who recover do not develop antitoxin in the blood.

2-Clostridium tetani

C. tetani, which causes **tetanus, is worldwide in distribution** in the soil and in the feces of horses and other animals. Several types of *C tetani* can be distinguished by specific flagellar antigens. All share a common **O (somatic) antigen** and all produce the same antigenic type of neurotoxin, tetanospasmin.

- **Toxin**

The vegetative cells of *C tetani* produce the toxin **tetanospasmin** (MW, 150,000) that is cleaved by a bacterial protease into two peptides (MW, 50,000 and 100,000) linked by a disulfide bond. **T**he toxin initially binds to receptors on the presynaptic membranes of motor neurons. It then migrates by the retrograde axonal transport system to the cell bodies of these neurons to the spinal cord and brainstem.

Release of the inhibitory glycine and GABA is blocked, and the motor neurons are not inhibited. Hyperreflexia, muscle spasms, and spastic paralysis result.

- **Clinical Findings**

The incubation period may range from 4 to 5 days to as many weeks. The disease is characterized by tonic contraction of voluntary muscles. the muscles of the jaw which contract so that the mouth cannot be opened. The patient is fully conscious, and pain may be intense. Death usually results from interference with the mechanics of respiration. The mortality rate in generalized tetanus is very high.

3-Clostridia That Produce Invasive Infections

Many different toxin-producing clostridia (*C.perfringens and* related clostridia) can produce invasive infection (including **myonecrosis and gas gangrene)** if introduced into damaged tissue. About 30 species of clostridia may produce such an effect, but the most common in invasive disease is ***C perfringens*** (90%). An enterotoxin of *C perfringens* is a common cause of **food poisoning.**

- **Toxins**

The invasive clostridia produce a large variety of toxins and enzymes that result in a spreading infection. Many of these toxins have lethal, necrotizing, and hemolytic properties.

The **α toxin** of *C perfringens type A is a* lecithinase, and its lethal action is proportionate to the rate at which it splits lecithin (an important constituent of cell membranes) to phosphorylcholine and diglyceride. Some strains of *C.perfringens*

produce a powerful **enterotoxin**, especially when grown in meat dishes. When more than 108 vegetative cells are ingested and sporulate in the gut, enterotoxin is formed. It induces intense diarrhea in 7–30 hours. **The action** of *C perfringens* enterotoxin involves marked hypersecretion in the jejunum and ileum , with loss of fluids and electrolytes in diarrhea.

- **Clinical Findings**

From a contaminated wound ,the infection spreads in 1–3 days to produce crepitation in the subcutaneous tissue and muscle, foul-smelling discharge, rapidly progressing necrosis, fever, hemolysis, toxemia, shock, and death.

Treatment is with early surgery (amputation) and antibiotic administration. early amputation was the only treatment. At times, the infection results only in anaerobic fasciitis or cellulitis.

4-Clostridium difficile and Diarrheal Disease

Pseudomembranous Colitis

Pseudomembranous colitis is diagnosed by detection of one or both *C difficile toxins* in stool and by endoscopic observation of pseudomembranes or micro abscesses in patients who have diarrhea and have been given antibiotics. The diarrhea may be watery or bloody, and the patient frequently has associated abdominal cramps, leukocytosis, and fever. Although many antibiotics have been associated with pseudomembranous colitis, the most common are ampicillin and clindamycin and more recently, the fluoroquinolones. Administration of antibiotics results in proliferation of drug-resistant *C difficile* that produces two toxins. **Toxin A**, a potent enterotoxin that also has some cytotoxic activity, binds to the brush border membranes of the gut at receptor sites. **Toxin B** is a potent cytotoxin.