Pathogenic Bacteria (practical)

Family: Bacillaceae

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Bacillaceae

Genus: *Bacillus*

- ***B. anthracis***
- ***B. cereus***
- ***B. subtilis***

General Characteristics: The genus *bacillus* includes large aerobic, gram-positive rods have square ends and are arranged in long chains; spores are located in the center of the nonmotile bacilli. 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. *B. cereus* can grow in foods and produce an enterotoxin or an emetic toxin and cause **food poisoning**. *B. anthracis,* which causes **anthrax,** is the principal pathogen of the genus. 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 (eg, hides, bristles, hair, wool, bone) can be sterilized by autoclaving.

Culture and Growth Characteristics: When grown on blood agar plates, the organisms produce non hemolytic gray to white round colonies with a rough texture and have a "cut glass" appearance in transmitted light. Comma-shaped outgrowths (Medusa head) may project from the colony. Hemolysis is uncommon with *B. anthracis* but common with the saprophytic bacilli. Carbohydrate fermentation is not useful. In semisolid medium, anthrax bacilli are always nonmotile, whereas related nonpathogenic organisms (eg, *B cereus*) exhibit motility by "swarming." Gelatin is liquefied, and growth in gelatin stabs resembles an inverted fir tree. The saprophytic bacilli utilize simple sources of nitrogen and carbon for energy and growth. Demonstration of capsule requires growth on bicarbonate-containing medium in 5–7% carbon dioxide.

Pathogenesis:

B. anthracis causes **anthrax:** In humans, 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).

B. cereus cause **Food poisoning,** eye infections, severe keratitis, endophthalmitis, panophthalmitis, localized infections and systemic infections, including endocarditis, meningitis, osteomyelitis, and pneumonia.

Specimens: Specimens to be examined are fluid or pus from a local lesion, blood, and sputum.

Laboratory diagnostic tests:

1. **Gram stains (**chains of large gram-positive rods).
2. **Blood agar**
3. **Pathogenicity in mouse:** Virulent anthrax cultures kill mice or guinea pigs upon intraperitoneal injection.
4. **Starch Hydrolysis: (*B. subtilis* is α-amylase positive)**

The starch molecule consists of two constituents: amylose, an unbranched glucose polymer and amylopectin, a large branched polymer. Both amylopectin and amylose are rapidly hydrolyzed by certain bacteria, using their α-amylases, to yield dextrins, maltose, and glucose, as follows:

Starch **α-amylase**

[Amylose + Amylopectin] ⟶ **Dextrins + Maltose + Glucose**

(Large polysaccharide) **H_2O** **(Intermediate Polysaccharides)** **(Disaccharide)** **(Monosaccharide)**

Gram's iodine can be used to indicate the presence of starch. When it contacts starch, it forms a blue to brown complex. Hydrolyzed starch does not produce a color change. If a clear area appears after adding Gram's iodine to a medium containing starch and bacterial growth, α-amylase has been produced by the bacteria. If there is no clearing, starch has not been hydrolyzed.

Propriety	***B. anthracis***	***B. cereus***	***B. subtilis***
Motility	**-**	**+**	**+**
Capsule	**+**	**+**	**-**
Optimal growth temperature	**37 ºC**	**30 ºC**	**37 ºC**
Pathogenicity in mouse	**+++**	**+**	**-**

Treatment: Ciprofloxacin, penicillin G, along with gentamicin or streptomycin. Some other gram-positive bacilli, such as *B. cereus,* are resistant to penicillin by virtue of β-lactamase production. Doxycycline, erythromycin, or ciprofloxacin may be effective alternatives to penicillin.

Family: Bacillaceae

Phylum: Firmicutes

Class: Clostridia

Order: Clostridiales

Family: Bacillaceae

Genus: *Clostridium*

C. botulinum
C. tetani
C. perfringens
C difficile

General Characteristics: 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**.** 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.

Culture and Growth Characteristics: the clostridia grow well on the blood-enriched media used to grow anaerobes and on other media used to culture anaerobes as well. Some clostridia produce large raised colonies (eg, *C. perfringens*); others produce smaller colonies (e.g, *C. tetani*). Some clostridia form colonies that spread on the agar surface. Many clostridia produce a zone of haemolysis on blood agar. *C. perfringens* typically produces multiple zones of haemolysis around colonies. Clostridia can ferment a variety of sugars; many can digest proteins. Milk is turned acid by some and digested by others and undergoes "stormy fermentation" (ie, clot torn by gas) (e.g, *C. perfringens*). Various enzymes are produced by different species.

Pathogenesis:

C. botulinum causes **botulism.**

C. tetani causes **tetanus.**

C. perfringens can produce invasive infection (including **myonecrosis** and **gas gangrene**) if introduced into damaged tissue. An enterotoxin of *C. perfringens* is a common cause of food poisoning.

C. difficile causes **Pseudomembranous Colitis**

Laboratory diagnostic tests:

1. ***C. botulinum:*** Toxin can often be demonstrated in serum from the patient, and toxin may be found in leftover food. Mice injected intraperitoneally die rapidly. The antigenic type of toxin is identified by neutralization with specific antitoxin in mice. *C botulinum* may be grown from food remains and tested for toxin

production, but this is rarely done and is of questionable significance. Toxin may be demonstrated by passive hemagglutination or radioimmunoassay.

2. ***C. tetani*** **:**

Specimens: wounds swb, exudates in tissue from wound, gram staining shows gram positive bacilli with drum-stick appearance.

Culture: Specimens are inoculated on the blood agar or on cooked meat medium under anaerobic conduction, *C. tetani* produce swarming growth after 1-2 days of incubation.

3. ***C. perfringens:***

Specimens: Specimens consist of material from wounds, pus, and tissue. The presence of large gram-positive rods in Gram-stained smears suggests gas gangrene clostridia; spores are not regularly present.

Culture: Material is inoculated into **chopped meat-glucose** medium and **thioglycolate** medium and onto **blood agar** plates incubated anaerobically. *C perfringens* rarely produces spores when cultured on agar in the laboratory.

Haemolysis on blood agar

Litmus milk reaction: The growth from one of the media is transferred into milk. A clot torn by gas in 24 hours is suggestive of *C. perfringens.*

biochemical reactions (various sugars in thioglycolate)

Lecithinase activity is evaluated by the precipitate formed around colonies on egg yolk media.

Final identification **rests on toxin production and neutralization by specific antitoxin.**

Litmus milk reaction:

Medium: **Litmus milk broth contains:**

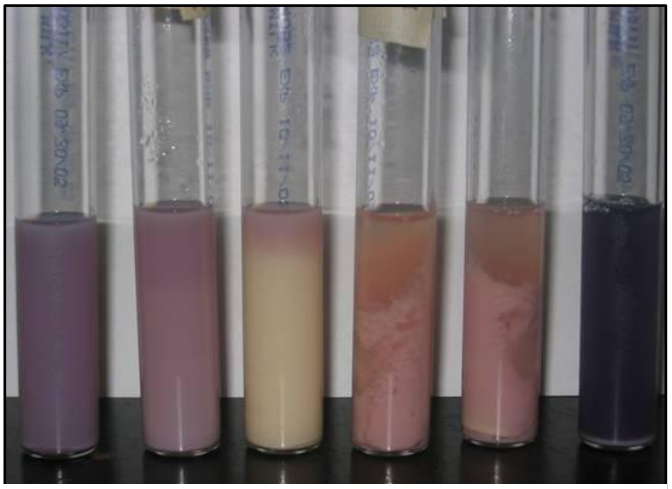
Milk that contains sugar lactose and protein casein
Litmus: as pH indicator (purple color turn to pink color in acidic pH and to blue in alkaline pH) and oxidation-reduction indicator.

Result:

1. **No color change**: remain purple, no change in the texture of the fluid.
2. **Acid production** (lactose fermentation as a result of production β-galactosidase enzyme): pink color
3. **Acid followed by reduction**: continued incubation may produce a white color at the bottom of the tube (reduced litmus) with a pink band at the top of the tube.
4. **Acid followed by reduction, followed by crud formation:** continued incubation may produce a crud at the bottom of the tube, as a result of produce a rennin enzyme from bacteria that causes casein to coagulate and form a rennet crud (clot) along with acid production and gas formation (CO_2 and/or H_2) and these gases may seen as separation of the crud, the presence of bubbles in the crud, or developed of tracts or

fissures in the crud. Some bacteria, such as clostridia, produce so much gas that the crud is torn to shreds. This is known as **stormy fermentation.**

5. **Proteolysis:** decrease in turbidity of milk due to loss of the colloid casein. Purple band at the top of the tube and a brown color throughout the rest of the tube. The milk also becomes watery.
6. **Alkaline reaction:** change in the color from purple to blue or dark blue as a result of decarboxylation or deaminationof the casein amino acids.

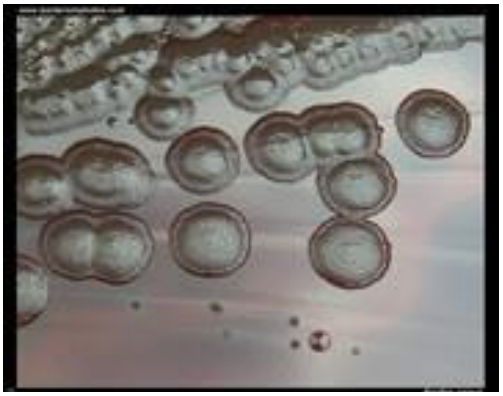


Control , pink=acid , white=reduction , stormy fermentation , blue=alkaline

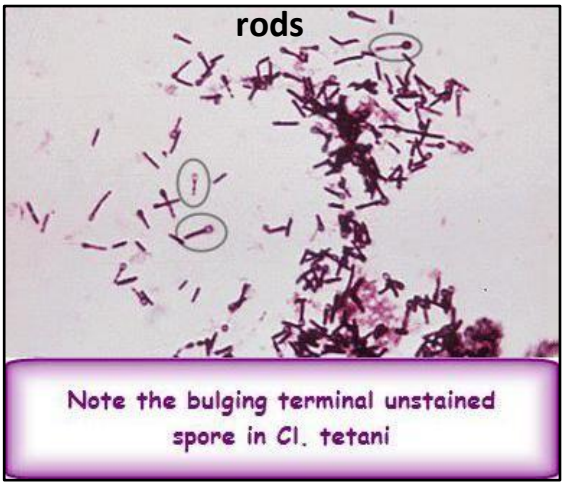
Litmus milk reaction

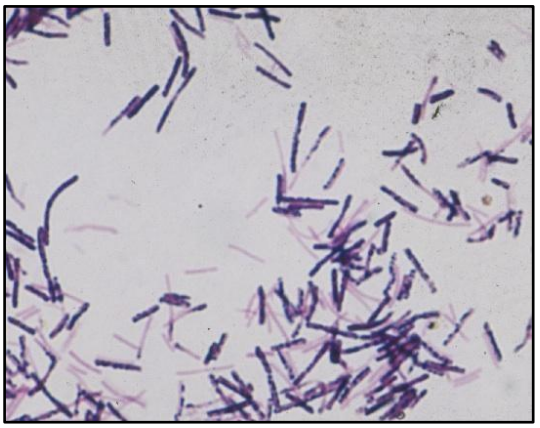
Colonies of on blood agar *B. cereus*

***Bacillus* chains of large gram-positive rods**



Colonies *B. cereus* of on blood agar





***Clostridium ssp.* gram-positive rods**