***Bacillus*:**

Introduction:

The family Bacillaceae consists of different types of bacteria. All of these are able to form **endospores**. The **two** clinically important genera are ***Bacillus*** (the **aerobic and facultative anaerobic spore-formers**) and ***Clostridium*** (the **strict anaerobic spore-formers**).

General characteristics of *Bacillus*

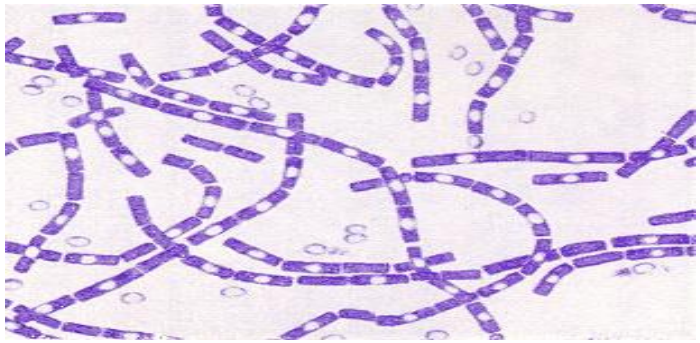
1. The genus *Bacillus* consists of **aerobic** bacilli forming heat resistant **spores**.
2. They are **gram-positive**.
3. They are generally motile with peritrichous flagella, (*bacillus anthracis* in non-motile).
4. Most form catalase and most produce acid but not gas from glucose.
5. The genus includes psychrophilic, **mesophilic** and **thermophilic** species.
6. Their salt tolerance varies from less than 2 to 25 percent NaCl.

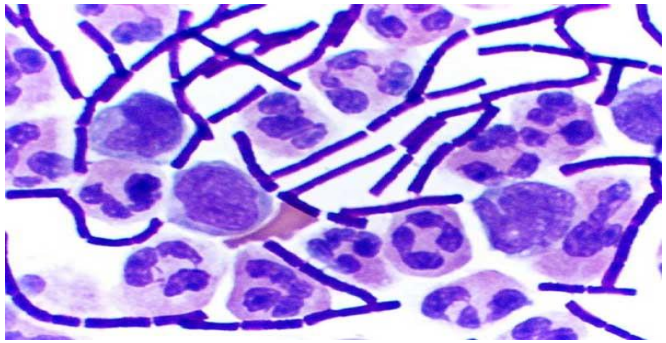
Morphology

B. anthracis is one of the largest of pathogenic bacteria (3 to 8 by 1 to 1.3 μm) and is **gram-positive**, **spore forming** *bacillus*. In cultures, the bacilli are arranged end to end forming **long chains**. The ends of the bacilli are concave and somewhat swollen so that the chain of bacilli presents is '**bamboo stick' appearance**.

The spore is **oval** , **central in position and of the same diameter as the *bacillus*, therefore, not swelling the mother cell**. Spores seen as unstained spaces in Gram-stained bacilli .

The cell or even the entire chain is surrounded by a **capsule** which is **polypeptide** in nature, being composed of a polymer of **D– glutamic acid.**





Cultural Characteristics

It is aerobe and facultative anaerobe. Temperature range for growth is 12-45°C (optimum 37°C). Optimum pH for growth is 7.4 .

1. **Nutrient agar:** On nutrient agar, colonies are irregularly round, 2-3 mm in diameter, raised, opaque, grayish white, with a frosted glass appearance. The edge of the colony is composed of long, interlacing chains of bacilli; resembling ‘Medusa head appearance’ as in figure below.

Medusa head appearance colonies

2. **Blood agar:** Colonies on horse or sheep blood agar are non-hemolytic, but some strains produce a narrow zone of hemolysis (finger like appearance).

3.**In broth**: Growth develops silky strands, a surface pellicle floccular deposit.

4.**In a gelatin stab**, there is growth down the stab line with lateral spikes, longer near the surface, .giving an 'inverted fir tree' appearance with slow lique¬faction commencing from the top

5.**Selective medium:** A selective medium for *B. anthracis* is (PLET medium, consisting of .5 polymyxin, lysozyme, ethylene diamine tetra acetic acid- EDTA) and thallous acetate added to heart infusion agar, is used to isolate *B. anthracis* from mixtures containing other spore-bearing bacilli.

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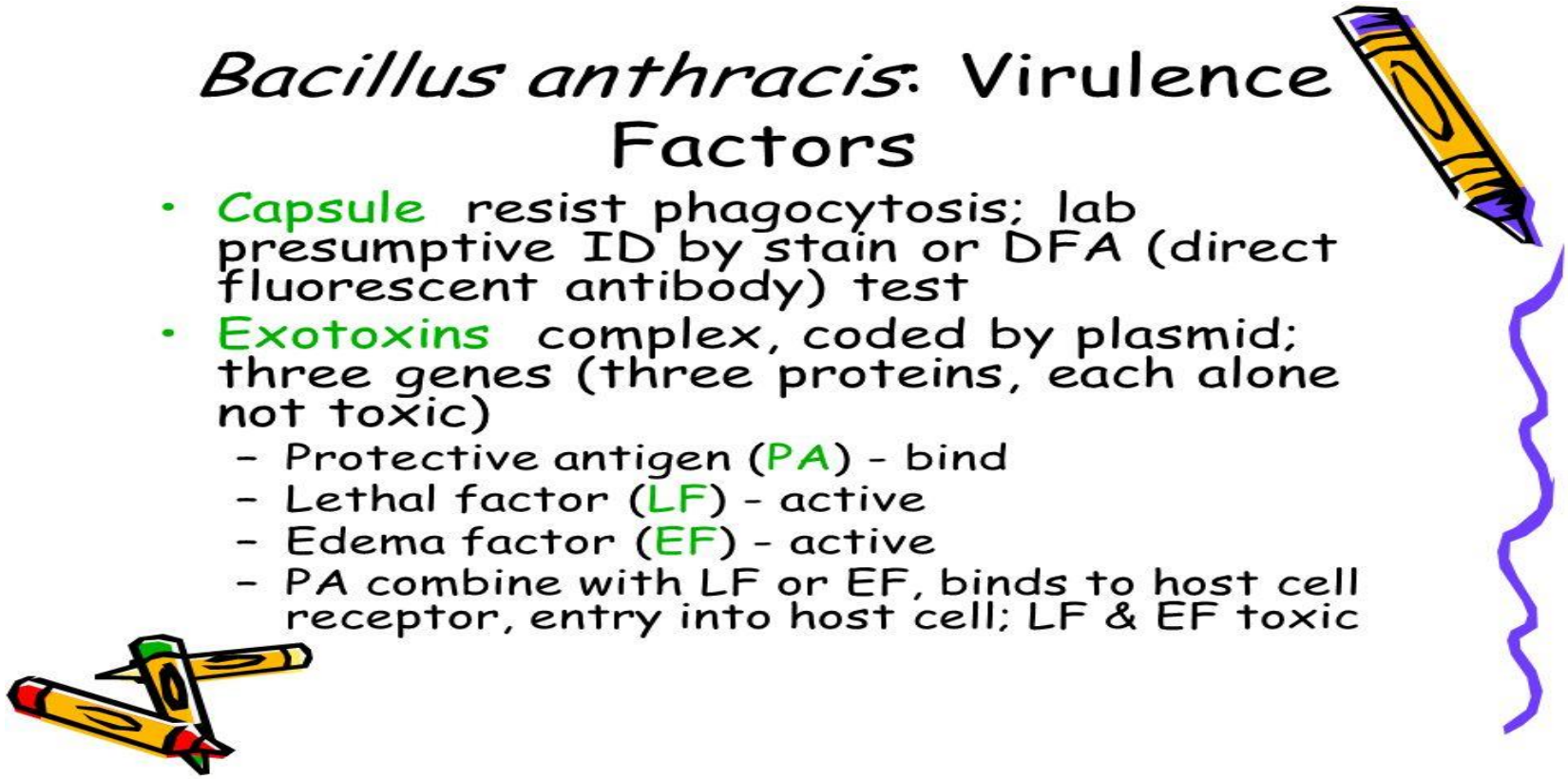
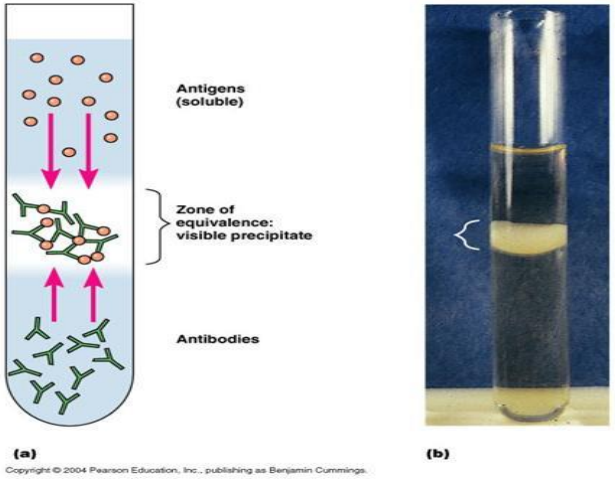
Biochemical Reactions: *B. anthracis* ferments glucose, maltose, sucrose, trehalose and dextrin with the production of acid without gas. Nitrates are reduced to nitrites. Catalase is formed. There is a weak lecithinase reaction on egg-yolk agar which gives a narrow zone of opalescence around the colonies



Anthrax: anthrax is a **zoonotic disease** (it is transmitted between animals and human). It is mainly of animal disease and transmits from animals to human by different ways causing **three types of anthrax disease depending on the site of entry**; either **cutaneous** anthrax (the common and treatable type), **pulmonary** anthrax (**wool's sorter disease**, fatal) or **gastrointestinal** anthrax (***see the diagram below, please***).

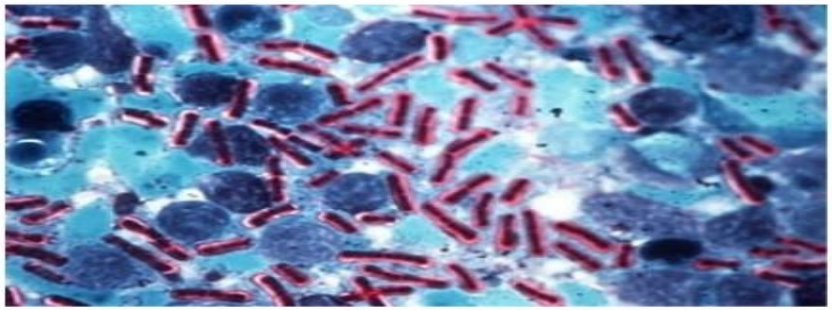
<u>Ascoli test:</u> it is serological, ring precipitin test using an extract of infected tissue and anthrax antiserum.



McFadyean reaction: It is special staining reaction, demonstrating a **pink capsule around a blue cell using polychrome methylene blue stain**.

B. anthracis

McFadyean reaction(polychrome MB stain)



Cutaneous anthrax on the neck

Treatment of *Bacillus anthracis*

Antibiotics are usually used to treat anthrax. Antibiotics that may be prescribed include penicillin, doxycycline, and ciprofloxacin. Inhalation anthrax is treated with a combination of antibiotics such as ciprofloxacin plus another medicine. They are given by IV (intravenously).

Bacillus cereus: it is a normal inhabitant of the soil, but it can be regularly isolated from foods such as grains and spices. *B. cereus* causes **two types of food-borne intoxications**. **One** type is characterized by **nausea**, **vomiting** and **abdominal cramps** and has an incubation period of **1 to 6 hours**. **This is the "short-incubation" or emetic form of the disease caused by (heat-stable emetic toxin)**. The **second** type causes **abdominal cramps and diarrhea** with an incubation period of **8 to 16 hours** . Diarrhea may be a small volume or profuse and watery. This type is referred to as the **"long-incubation" or diarrheal form of the disease caused by the heat-labile diarrhea-genic enterotoxin**. In either type, the illness usually lasts less than 24 hours after onset.

Treatment of *Bacillus cereus*

B. cereus produces beta-lactamases, unlike *Bacillus anthracis*, and so is resistant to beta-lactam antibiotics; it is usually susceptible to treatment with clindamycin, vancomycin, gentamicin, chloramphenicol, and erythromycin. Simultaneous therapy via multiple routes may be required.

Comparison between *B. anthracis* and *B. cereus*:

B. anthrax vs *B. cereus*

Sr. No.	Feature	B. anthrax	B. cereus
1	Pathogenicity	Anthrax	Food poisoning
2	Capsule	Poly D-glutamic acid	Absent
3	Motility	Non motile	Motile
4	Source of infection	Spores from animal products	Spores on grains and reheated fried rice
5	Portal of Entry	Skin, Respiratory tract,GIT	GIT
6	Virulence factors	Anthrax toxin, Capsule; inhibits phagocytosis and opsonization	Two types of enterotoxinss; one increases the conc. Of cAMP in gut and 2nd act as super Ag
7	Clinical features	Pinsless ulcers with black crust, edema, lesion, bacteremia, lymphadenitis	Nausea, vomiting, watery and non bloody diarrhea

Clostridium Bacteria

Introduction:

The genus *Clostridium* includes all anaerobic, gram-positive bacilli and capable of forming endospores. Spores of clostridia are usually wider than the diameter of the rods in which they are formed, giving the bacillus a swollen appearance resembling a spindle. The name *Clostridium* is derived from the word 'Kloster' (meaning a spindle).

General features of Clostridium:

1. Morphology

The clostridia are 1) gram-positive typically large, 2) straight or slightly curved rods, 3-8 × 0.6-1 μm with slightly rounded ends. Gram-positive, pleomorphism forms are common. 3) Most species of clostridia are motile with peritrichous flagella except *Cl. perfringens* and *Cl. tetani* type VI which are non-motile. All clostridia are non-capsulated with the exception of *Cl. perfringens*.

All clostridia produce endospores. 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, sub-terminally, or terminally.

2. Culture

Most species are obligate anaerobes. A few species grow in the presence of trace amounts of air and some actually grow slowly under normal atmospheric conditions. Clostridia grow on enriched media in the presence of reducing agent such as cysteine or thioglycollate (to maintain a low oxidation-reduction potential)

Liquid media like cooked meat broth (CMB) or thioglycollate media (containing reducing agent thioglycollate and 0.1% agar) are very useful for growing clostridia. A very useful medium is Robertson's cooked (RCM) meat broth. It contains unsaturated fatty acids which take up oxygen.

a-*Clostridium perfringens*: Gas gangrene

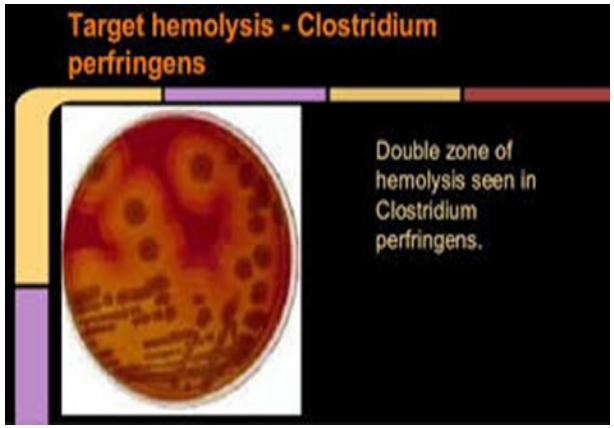
Morphology: It is a relatively large gram-positive bacillus (about 4-6 × 1 μm) with straight, parallel sides and rounded or truncated ends, occurring singly or in chains or small bundles. It is pleomorphic, capsulated and non-motile.

Spores are typically oval, central or sub-terminal and not bulging but are rarely seen in artificial culture.

Cultural Characteristics : It is an anaerobe but can also grow under microaero¬philic conditions. It grows over a pH range of 5.5 to 8.0 and temperature range of 20°C to 50°C

(optimum temperature range 37-45°C). Robertson's cooked meat broth inoculated with mixtures of *Cl.perfringens* and other bacteria and incubated at 45°C for 4 to 6 hours serves as enrichment. Blood agar plates streaked and incubated at 37°C will have proportionally higher numbers of *Cl. perfringens* (yield pure or predominant growth of *Cl. perfringens*). Good growth occurs in Robertson's cooked meat medium.

It grows best on carbohydrate-containing media such as glucose blood agar. Surface colonies are large, smooth, regular, convex, slightly opaque disks. Colonies of most strains demonstrate a 'target hemolysis, double zone of hemolysis' after overnight incubation on rabbit, sheep, ox, or human blood agar. It results from a narrow zone of complete hemolysis due to theta toxin and a much wider darker zone of incomplete hemolysis due to the α-toxin. On longer incubation this double zone pattern of hemolysis may fade.



Biochemical Reactions

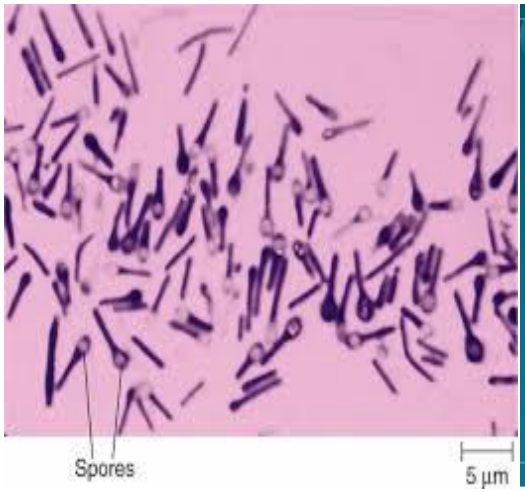
It is actively saccharolytic. Glucose, maltose, lactose and sucrose are fermented with the production of acid and gas. It is indole negative, MR positive and VP negative. Hydrogen sulfide (H2S) is produced abundantly; sulfite is actively reduced; most strains reduce nitrates to nitrites.

Treatment

Treatment must begin immediately. High doses of antibiotics, typically penicillin and clindamycin, are given, and all dead and infected tissue is removed surgically. About one of five people with gas gangrene in a limb requires amputation.

b- *Clostridium tetani*: Tetanus disease

Morphology: It is a gram-positive, slender bacillus, 2 to 5 × 0.4-1 mm with rounded ends. The spores are spherical, terminal and twice the diameter of vegetative cells giving them typical drumstick appearance. It tends to be pleomorphic and sometimes filamentous. It is non-capsulated and motile by peritrichous flagella. Young cultures of the organism usually stain gram-positive, but in older cultures and in smears made from wounds, they are Gram variable and even be gram-negative.



Cultural Characteristics

Cl. tetani is an obligate anaerobe. The optimal temperature for growth is 37°C, and the optimal pH is 7.4. It can grow well in cooked meat broth (CMB), thioglycollate broth, nutrient agar and blood agar. In cooked meat broth (CMB), growth occurs as turbidity and there is also some gas formation. The meat is not digested but becomes black on prolonged incubation. On blood agar the bacilli produce a swarming growth. On horse blood agar, the colonies of *Cl. tetani* are surrounded by a zone of α-hemolysis, which subsequently develops into β-hemolysis, due to the production of an oxygen-labile hemolysin known as tetanolysin. On egg-yolk agar, it does not produce opalescence or pearly layer.

Biochemical Reactions

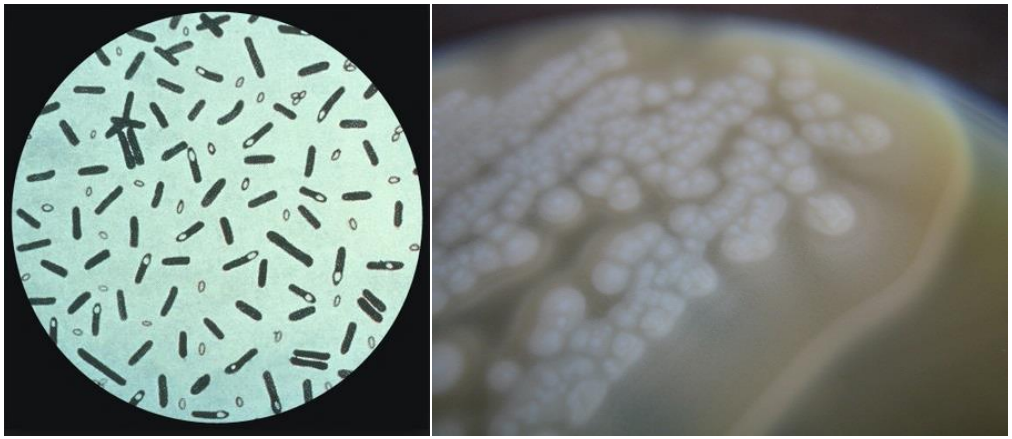
Cl. tetani has feeble (weak) proteolytic but no saccharolytic property. It does not attack any sugar. Gelatin liquefaction occurs very slowly. It is indole positive and MR, VP, H2S and nitrate reduction negative. A greenish fluorescence is produced on media containing neutral red (as on MacConkey's medium).

Treatment

These agents are used to eradicate clostridial organisms in the wound, which may produce tetanus toxin. They are administered to patients with clinical tetanus; however, their efficacy is questioned. Penicillin G was long considered the drug of choice, but metronidazole is now considered the antibiotic of choice

c- Clostridium botulinum

C. botulinum (from the Latin botulus, “sausage”) causes botulism. Botulism is a severe, often fatal, form of food poisoning characterized by pronounced neurotoxic effects. The disease has been caused by a wide range of foods, usually preserved hams, sausages, home-preserved meats and vegetables, canned products such as fish, liver contact with the organism itself is not required; hence the disease can be a pure intoxication.



Morphology: *C. botulinum* is a strictly anaerobic gram-positive bacillus (about 5 x 1 mm). It is non-capsulated, motile with peritrichous flagella and produces spores which are oval, sub-terminal and bulging.

Cultural Characteristics: It is a strict anaerobe. Optimum temperature is 35°C but some strains may grow even at 1 to 5°C. Good growth occurs on ordinary media. Surface colonies are large, irregular, and semi-transparent, with fimbriate border. On horse blood agar, all strains except those of type G are beta-hemolytic. On egg-yolk agar (EYA) all types except G produce opalescence and a pearly effect (as in above photo)

Resistance: Spores are heat and radiation resistant, surviving several hours at 100°C and for up to 10 minutes at 120°C. Spores of non-proteolytic types of B, E and F are much less resistant to heat. The resistance of the spores to radiation is of special relevance to food processing.

Treatment

In addition to that described, guanethidine and 4-aminopyridine have been used for the treatment of botulinum paralysis but have not been shown to be effective. The use of local antibiotics such as penicillin G or metronidazole may be helpful in eradicating *Clostridium botulinum* in wound botulism.