بسم الله الرحمن الرحيم

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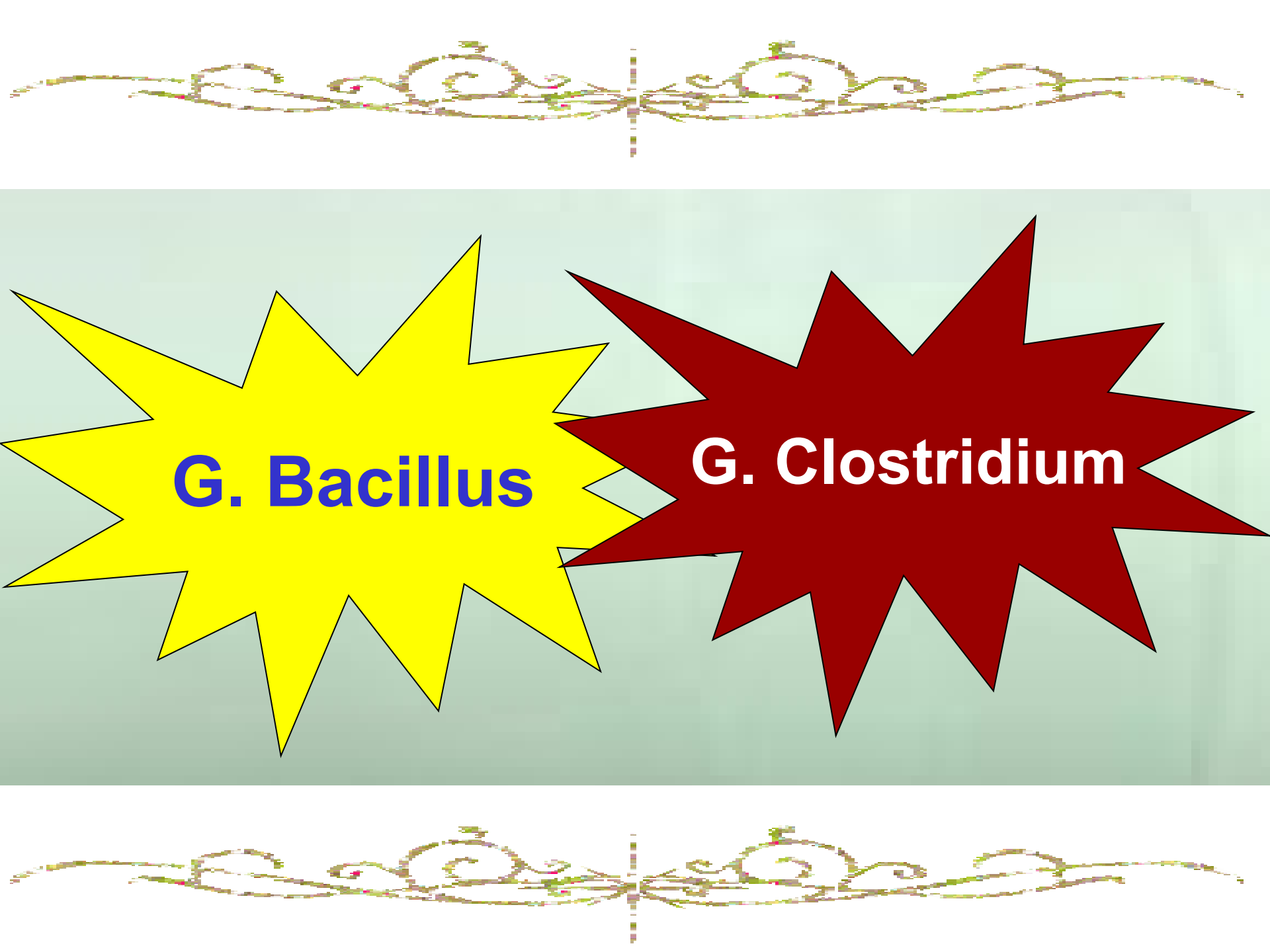
F. Bacillaceae


G. Bacillus
G. Clostridium

G. Bacillus	G. Clostridium
Aerobic	Obligatory anaerobic
Catalase +ve	Catalase -ve
Spore diameter equal to bacilli width (non bulging)	Spore diameter > bacilli width (bulging)

General Characteristics of G. Bacillus

- ~ 60 species; Gram-positive bacilli
  - Large (0.5 x 1.2 to 2.5 x 10 um)
  - Most are saprophytic contaminants or normal flora
  - *Bacillus anthracis* is the most important member.
- Produce endospores (not bulged)
- Aerobic or facultative anaerobic
- Catalase positive: Rapidly differentiates from *Clostridium*
- *Bacillus* spp. are ubiquitous
  - Soil, water, and airborne dust
- Thermophilic ($\leq$ 75°C) and psychrophilic ($\geq$5-8°C)
- Can flourish at extremes of acidity & alkalinity (pH 2 to 10)
- All members form rhizoid colonies

Genus:Bacillus

Bacillus anthracis
(Anthrax bacillus)

Bacillus anthracoides

(Pseudo Anthrax bacilli)

General Characters and Diseases of B. anthracis:

- *Bacillus anthracis* is a commonly found in soil of grazing areas.
- It is the only obligate pathogen within the genus Bacillus.
- *B. anthracis* is a large, straight (3-8×1-1.2μm), Gram positive, aerobic, spore-forming bacilli with square end (box-car or bamboo stick appearance) that arranged in short chains (two to a few cells) in blood or tissue smears from infected humans or animals, or long chains when grown in laboratory media.
- It is non motile.
- Spores are central, oval and non-bulged.
- Capsulation: unlike most other bacteria which have polysaccharide capsules, *B. anthracis* is capsulated by polypeptide (poly-D-γ-glutamic acid). Capsule appears *in-vivo* (blood, body fluid) or *in-vitro* in media containing bicarbonate and/ or 10% animal protein as defibrinated blood, serum and incubated at 10-20% CO_2 tension.

- It is non-haemolytic or (very rarely) only very weakly haemolytic on sheep or horse blood agar.
- It is sensitive to G penicillin.
- It is sensitive to the diagnostic ‘γ-phage: phage has the ability to lyse B. anthracis grown aerobically on blood or other nutrient agar and rarely lyses any other Bacillus species.
- *Bacillus anthracis* is the causative agent of anthrax in virtually all warm-blooded animals.

Pathogenicity: virulence factors

Two virulence factors –

- Capsular polypeptide – inhibits phagocytosis, encoded by a plasmid
- Anthrax toxin complex : made up of 3 fractions

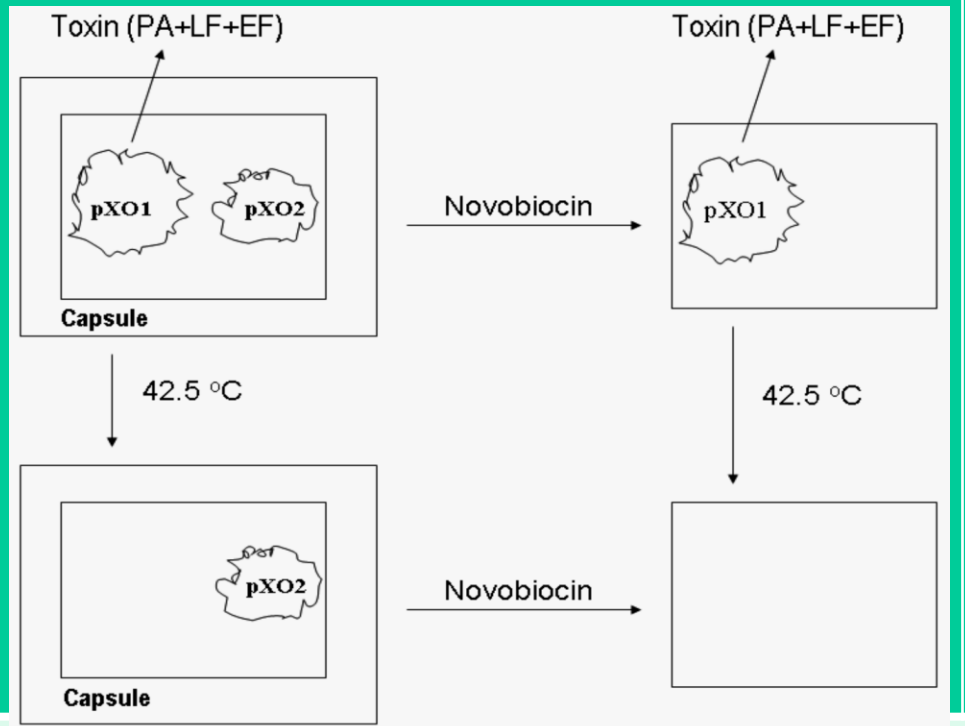
1. Edema factor (EF or Factor I)
2. Protective antigen factor (PA or Factor II)
3. Lethal factor (LF or Factor III)

* They are not toxic individually, the whole complex produces local edema & generalized shock.

N.B. The lethal toxin (PA+ LF), The edema toxin(PA+ EF)

* Toxin production and capsulation are plasmid mediated by pXO1 and pXO2 plasmids). Both the pXO1 and pXO2 plasmids are required for full virulence.

- The pXO1 plasmid contains the genes that encode for the 3 components of anthrax toxins.
- The pXO2 plasmid contains the genes that encode for the capsule synthesis.
- **N.B:** Loss of either plasmid pXO1 or pXO2, results in considerable reduction in (though not complete loss of) virulence and this has been the basis of anthrax vaccines since the end of the 19th century.



Diagrammatic representation of plasmids of B. anthracis and the results of their selective curing. Top left: fully virulent cell producing both toxin complex and capsule. Top right: curing of pXO2 results in the Sterne-type vaccine strain. Bottom left: curing of pXO1 results in the Pasteur-type vaccine strain. The bacterium can be cured of both plasmids (bottom right).

Anthrax

Acute fatal septicemia that affects all warm-blooded animals mainly herbivorous animals that cause:

- Cutaneous form
- Pulmonary form
- Severe enteritis

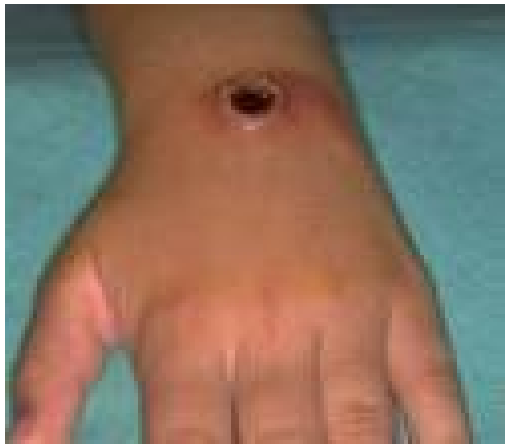
Susceptibility

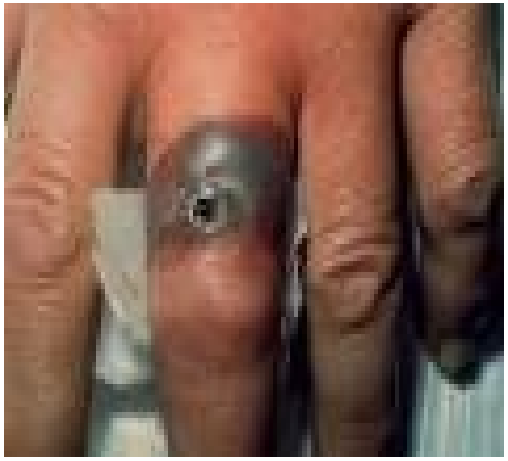
- Herbivorous animals (cattle, sheep “except Algerian sheep”, horse, camel and elephant)
- Pigs are intermediate
- Carnivore are rare
- Birds are very rare
- Humans almost invariably contract the natural disease directly or indirectly from animals or animal products. Anthrax is not transmitted from person to person.

- In cattle and sheep: Sudden death, swollen spleen, animal die within 1-2 days with no symptoms or after 4 days with acute septicemia, fever, decrease milk secretion, hemorrhagic unclotted blood from all natural orifices.

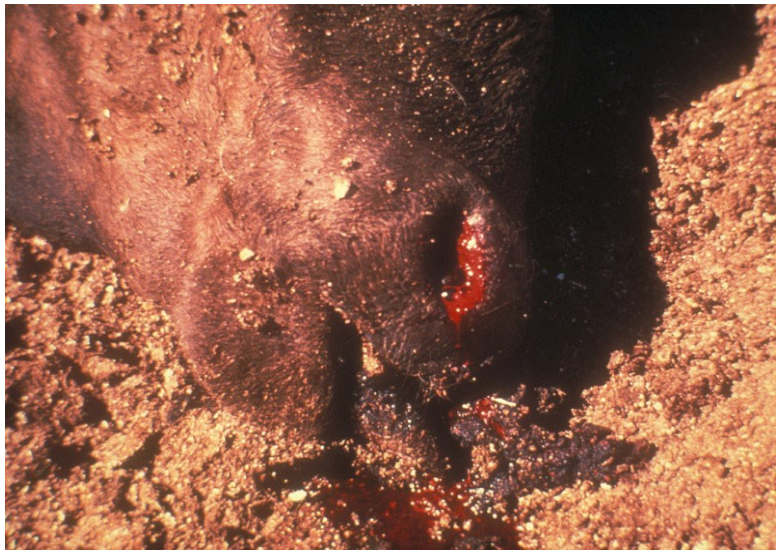
- Human: Three forms of human anthrax are recognized based on their portal of entry.

* All types lead to fatal septicemia





Cutaneous form of B.anthracis



Cattle showing tarry watery blood from natural orifices

orse

Throat oedema

Oedema at external genetalia

Pig

Throat oedema, septicemia

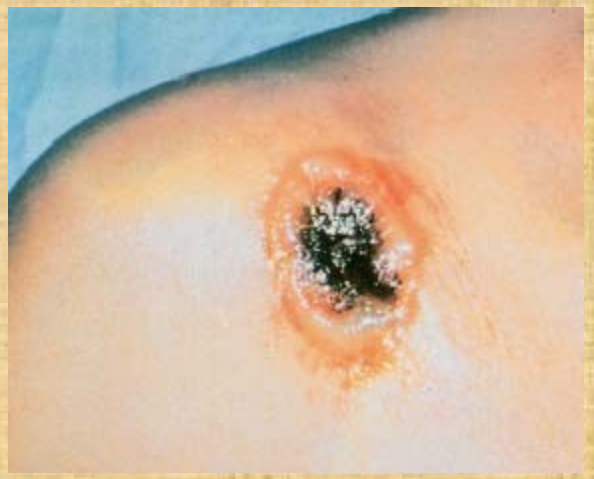
Dog, cat

Oedema and severe enteritis

Resistance

- Vegetative form die at 55-65°C
- Spore form is very resistant (die at 100for 10 minutes)

1. Cutaneous Anthrax



- The most common form

(95 % of human cases of anthrax)

- Route of entry: Skin
- Sites involved – face, neck, hands, arms & back
- Papule → Vesicles containing colorless or blood stained fluid → Malignant Pustule
- 'Malignant pustule' – satellite lesions filled with serum or yellow fluid arranged around a central necrotic lesion which is covered by a **black eschar**
- Also known as 'Hide Porter's disease'
- Mortality may reach 20% if untreaed due to fatal septicemia or meningitis

2. Pulmonary Anthrax

- Also called 'Wool Sorter's disease' – common in workers in wool factories by inhalation.
- A life-threatening hemorrhagic pneumonia caused by Inhalation of spores (95% mortality).

3. Gastrointestinal Anthrax

- Rare
- By ingestion of inadequately cooked meat containing *B. anthracis* spores
- Mortality may reach 20%.

Laboratory Diagnosis

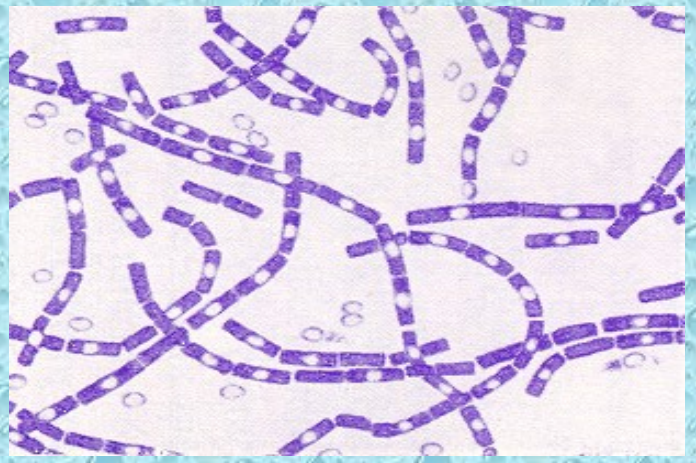
- Specimen: Contraindicated to open the carcasses. Just M.O. find air, it sporulated, resist dryness and hotness up to 50 years.
- Freshly dead animal: Blood from tail vein or ear vein.
- Old samples: Part of hide, wool, hair or any horney materials.

Morphology

- Gram +ve large bacilli (3-8µx1-1.2µ)
- Straight rods with square end that arranged in chains
- Sporulated by central non bulged spores (formed in culture at 32-34°C under aerobic condition)
- Capsulated by polypeptide capsule that found in blood, body fluid and media containing animal protein, serum, 10-25% CO_2.
- It can be stained by polychrome M.B. ⇒ Red capsule surrounding blue bacilli (Macfadyean´ reaction)
- Special stains are used to stain spores and capsule.

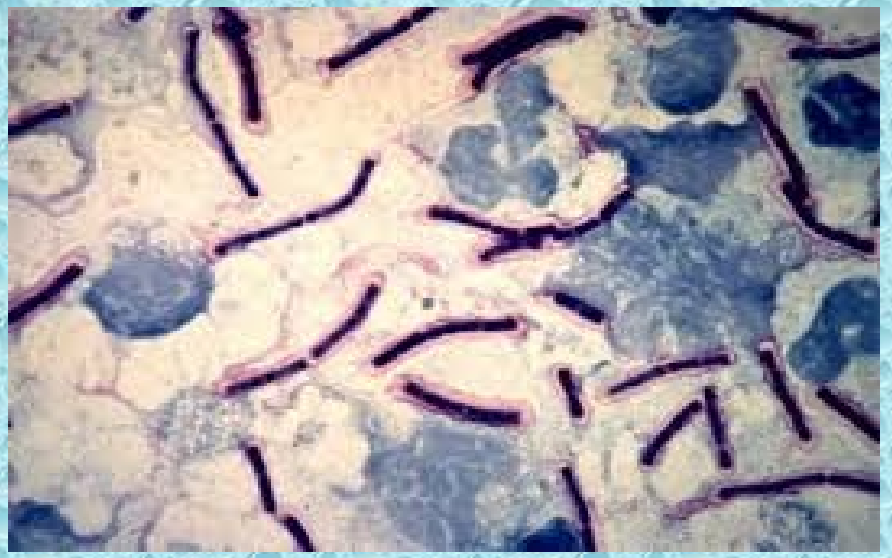
B. anthracis from culture, Gram stain

Central oval non bulging spore



Blood smear, Polychrome MB

Macfadyean´s reaction of B. anthracis



Culture charcters

- Grow on all ordinary media
- Aerobic and facultative anaerobic
- Grow at 12-43°C , best degree at 37°C
- Grow at slightly alkaline media (7.5-7.8)

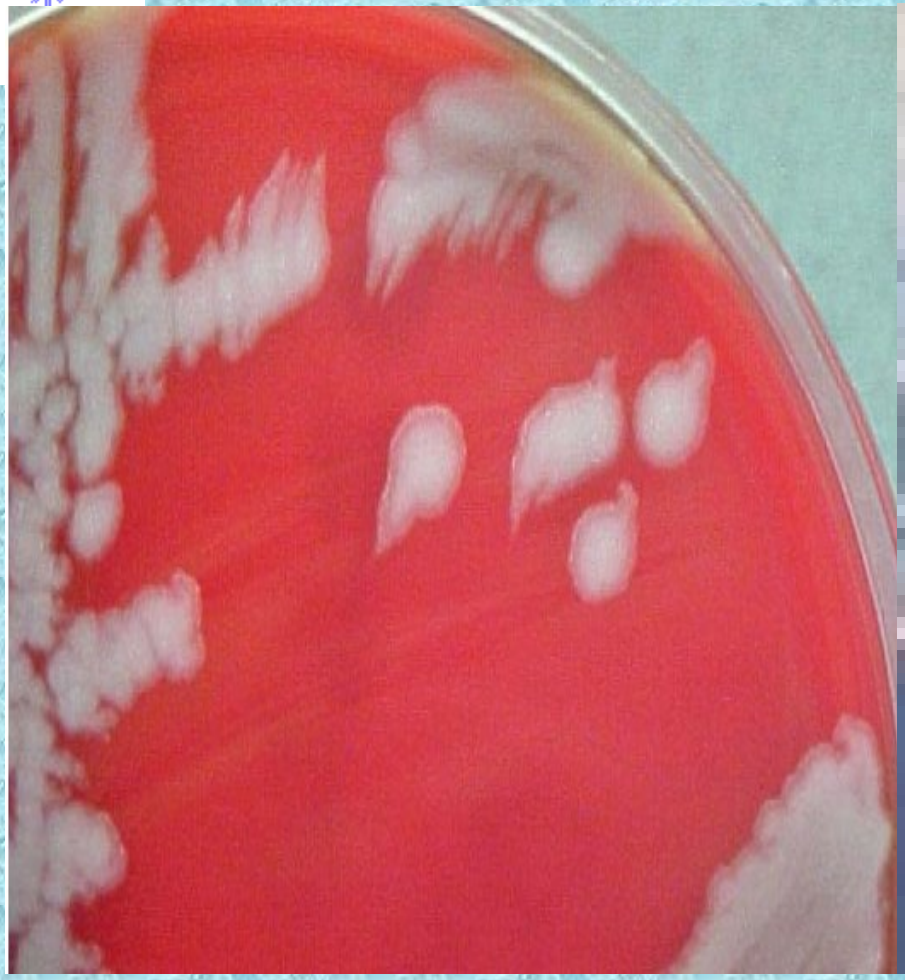
- **Nutrient agar:** Colonies are 2-3mm in diameter, dull, opaque, rought, grayish white with irregular outline that appear as medusa head or curled hair
- **Broth cultures:** floccular growth; due to the tendency of *B. anthracis* to form long chains, and granular deposit (cotton wool like appearance)
- **Gelatin medium:** Very characteristic type of liquefaction in which lateral line of growth produced from a central one the whole resemble inverted fir tree.

- **Blood agar:** no or narrow zone of haemolysis.

- **Selective media (PLET agar):** It is needed for the isolation of *B. anthracis* from clinical materials or environmental samples heavily contaminated with other bacteria. The best selective system was the polymyxin, lysozyme, EDTA and thallous acetate (PLET) agar. Its disadvantage lies in the ingredient, thallous acetate, which is highly toxic. Colonies are usually smaller in size on this medium compared to those on nutrient or blood agar. Medusa head are not seen.

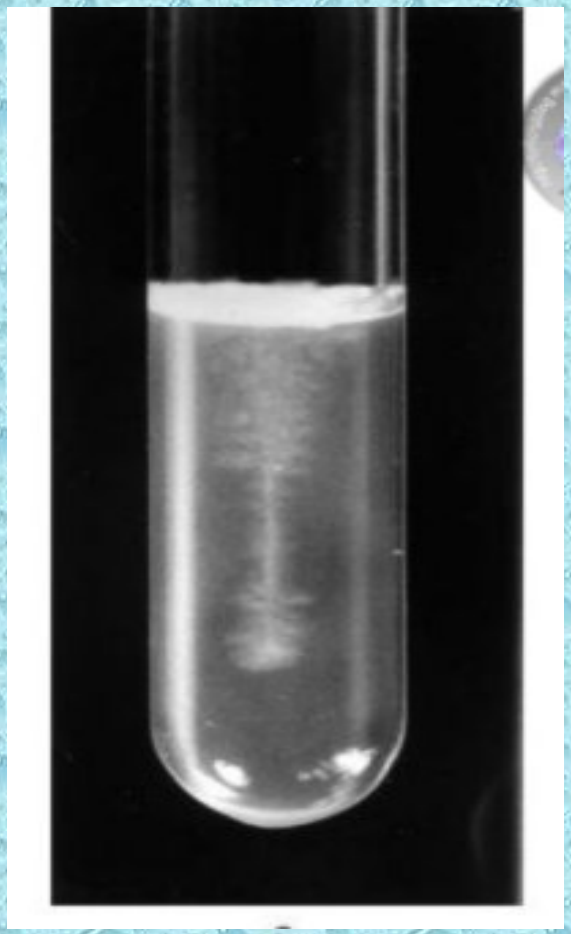
"String of Pearls reaction" on solid medium containing 0.05-0.5 units of Pn/ml, in 3-6 hrs the cells become large, spherical and occur in chains on agar surface, resembling a string of pearls.

- differentiates *B. anthracis* from *B. cereus*



B. anthracis **show no haemolysis on blood agar, medusa head**

String of Pearls reaction



Inverted fir tree

ochemical reaction

- It ferment glucose, maltose, sucrose, trehalose acid no gas
- Salicin suger fermentation late and slowly
- Starch hydrolysis
- Catalase +ve
- Nitrate +ve
- Litmus milk +ve
- Gelatin liquefaction +ve
- H2S -ve

Toxins

Lethal toxin **Oedema toxin**

Experimental infection (animal pathogenicity)

- Mice, guinea pig, rabbit while rat is resistant
- S/C injection resulted in death within 12-30 hours with sighs of septicemia.
- Dark red or tarry watery uncoagulated blood from all natural orifices
- Spleenomegaly, dark, friable liver, hepatomegaly
- gelatinous oedema at site of injection
- Blood smears prepared from dead animals→bacilli are seen in blood

Serological tests

Ascoli`s test

(Ring precipitation test)

(Thermo precipitation test)

It depends on the detection of polysaccharide Ag of *B. anthracis* in examined sample.

Samples: scraped hide, wool, hair, skin or piece of tissue

1- Boiling of 2cm of sample in 10 ml of sterile saline containing few drops of 0.5% acetic acid for10 min. then cool and filter.

2- In capillary tube, add 0.5 ml of filtered Ag to equal volume antianthrax serum

+ve result is ppt ring at junction between two fluids

uorescent Ab technique

+ve result is greenish illumination

Molecular identification

PCR depends on the unique specificity of the toxin and capsule and their genes.

Rapid immunoassays

for detection or confirmation of identity of *B. anthracis* are available commercially by using monoclonal antibodies against the anthrax protective antigen (PA).

Immunity (vaccination)

Passive immunity

Hyperimmune serum

Active immunity

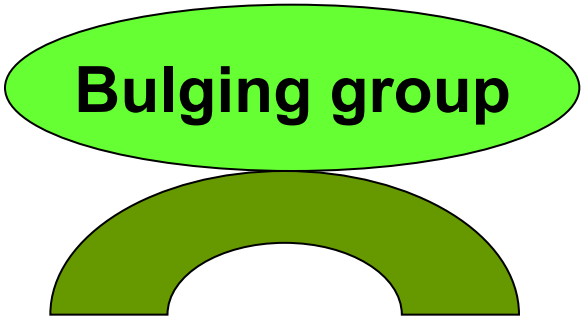
- Pasteur 1
- Pasteur II
- Spore vaccine
- Carbozoo vaccine
- Simultaneous

B.anthracoides

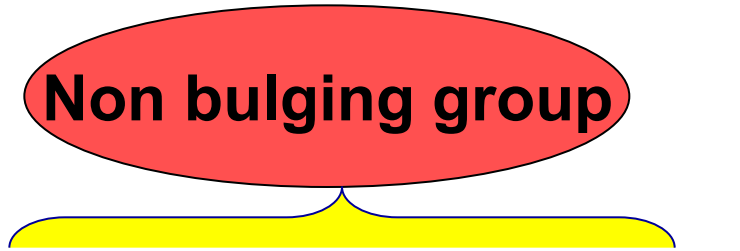
Saprophytic M.O. that widely distributed in nature (soil, water, dust and air). They are divided into 21 species.

According to spore

It may be:



B. polymexa ***B. circularis***



Large cells

B. cereus

B. mycoides

B. Mesentricous

B. megatherium

Small cells

B. subtilis

B. pumilis

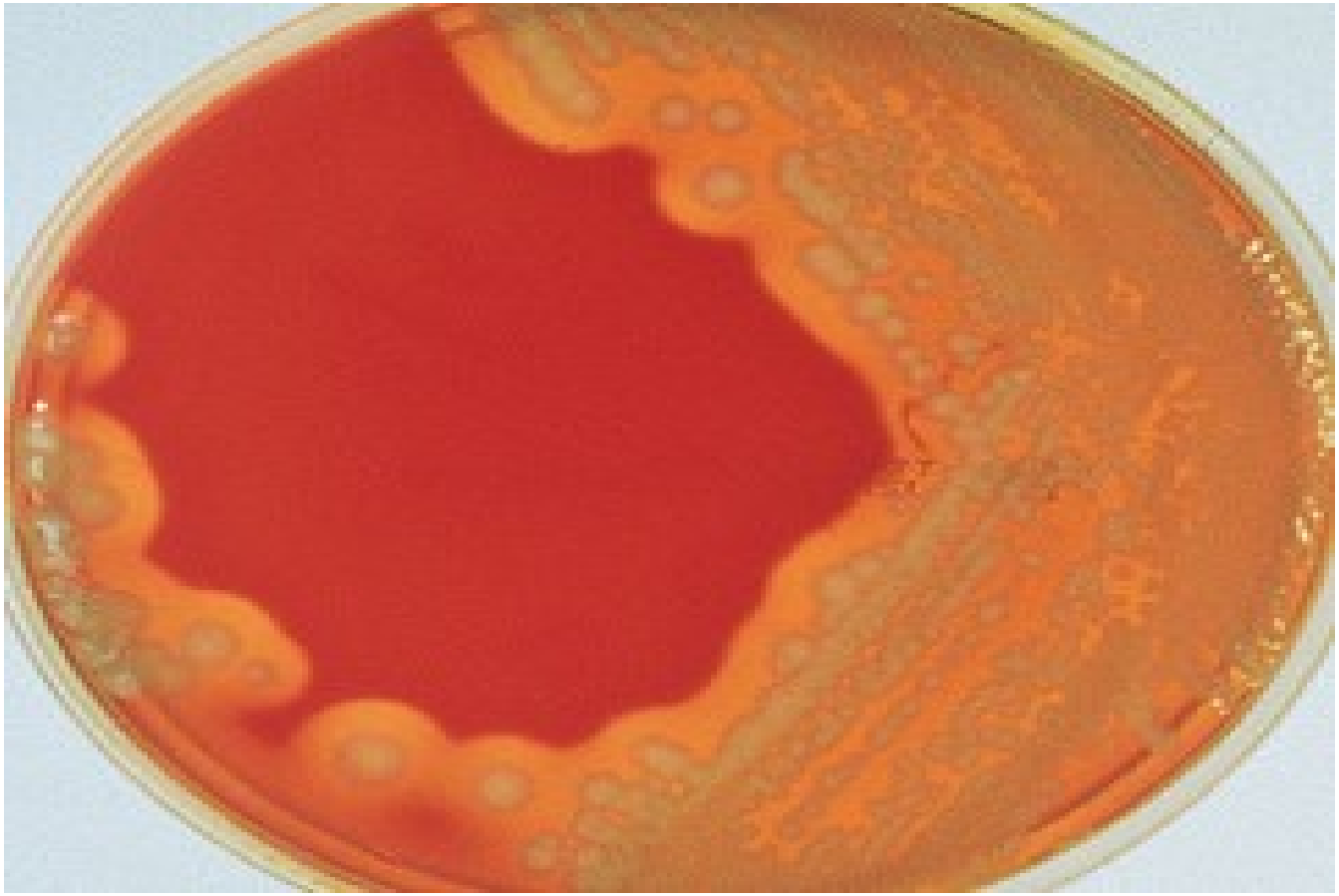
B. coagulans

orphology

- Gram +ve rods, rounded end, arranged in chains
- size (3-9μ x 0.4 – 1.2μ)
- Motile by 4-12 peritrichous flagella
- Spore is terminal or subterminal with or without bulging
- Non capsulated

Culture characters

- Ordinary media: Spreading growth
- Fluid medium: Pellicle or turbidity
- Gelatin media: fir tree
- Blood agar: β-haemolysis



Bacillus cereus **on sheep blood agar showing β-haemolysis**

Biochemical reactions

Sugar fermentation: ferment glucose, maltose and sucrose ⟹ acid only

Salicin sugar fermentation ⟹ rapid

Catalase test: +ve

Indole test: +ve

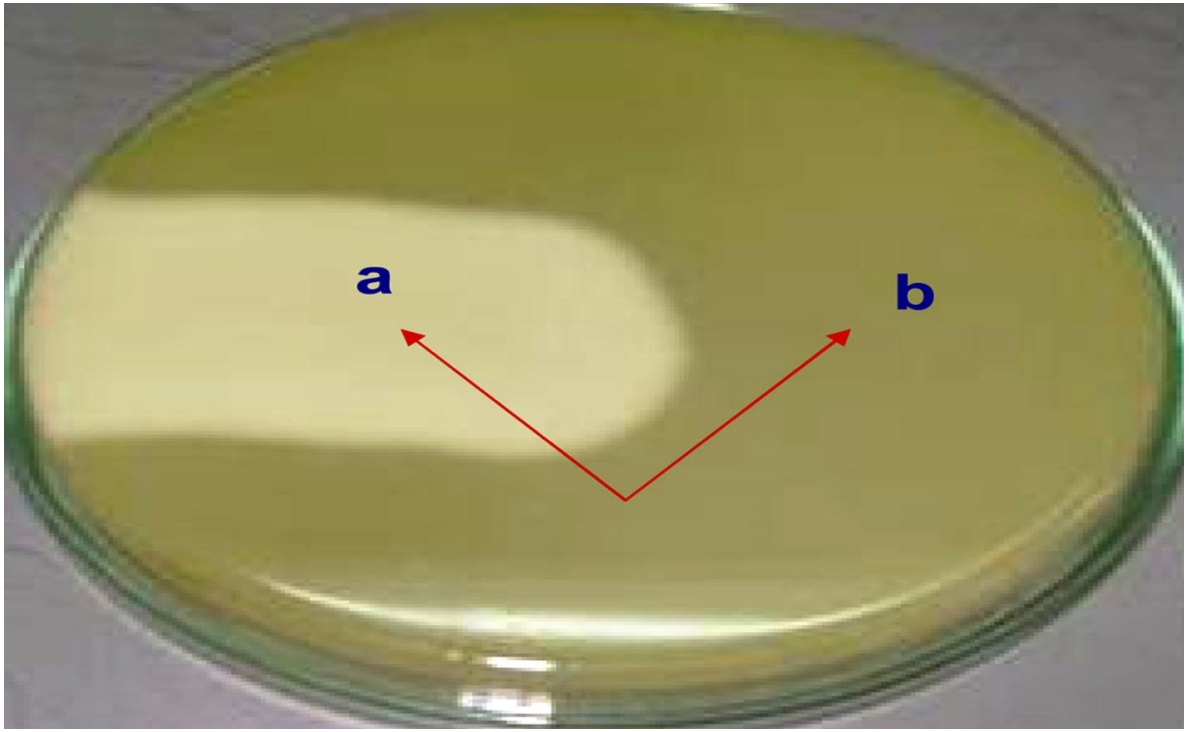
Complete reduction of methylene blue milk.

Nagler´s reaction

On lactose egg yolk milk agar medium

B. cereus and B. mycoides give +ve result that characterized as pearly opalescence zone surround the colony due to lecithin hydrolysis

Non lactose fermenter



a) Nagler´s reaction +ve

b) Nagler´s reaction -ve

Pathogenicity

Non pathogenic except:

B. cereus, B. subtilus, B. mycoides, B. polymyxa cause gastroenteritis and food poisoning

B. subtilis: severe eye infection

Resistance

Resist 121°C for an hour

Difference between *B. anthracis* and *B. anthracoides*

	B. anthracis	*B. anthracoides*
Pathogenicity	Highly pathogenic	Non or less pathogenic
Motility	Non motility	Motility
Capsule	Capsulated	Non capsulated
Growth at 45˚C	No growth	Growth occurs
In nutrient broth	Fluffy Cotton wool without pellicle	Turbidity and pellicle but no fluffy Cotton wool
Stabbing in gelatin	Inverted fir tree	Fir tree
liquefaction Of gelatin	Occurs slowly from up to down	Liquefy gelatin rapidly
On sheep blood agar	Non-haemolytic	β-haemolytic
Nagler´s reaction	Weak and slow	Strong and rapid
Penicillin agar media 10Mg\ml	No growth	Growth occurs rapidly
Effect of γ-phage	Lysis	Not lysis