

Bacillus anthracis
anthrax

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zoonotic disease unit
post graduate lecture

Family: Bacillaceae

Genus: Bacillus

The bacillus species divided into:

Bacillus cereus group:

- 1 .*B. anthracis* pathogenic
- 2 .*B. cereus* pathogenic
- 3 .*B. thuringiensis* pathogenic
- 4 .*B. mycooides*

other bacillus group:

1. *B. subtilis*
2. *B. licheniformis*
3. *B. firmus*
4. *B. coagulans*
5. *B. circulans*

*The word anthrax is derived from a Greek word meaning charcoal or **carbuncle** because of the ulcers with dark center.*

Another name of anthrax:

Splenic Fever, Carbon, Wool Sorters Disease, Malignant Pustule (In Human).

Main diseases of *Bacillus* species:

1. *B. anthracis* causes anthrax in all mammalian species & human but birds are highly resistance.
2. *B. cereus* produces Entero toxin that is responsible for food poisoning in human & rare infections in animals.
3. *B. piliformis* is responsible for Tyzzer's disease in laboratory mice. This disease is acute fatal infection causing hepatitis, enteritis & colitis.
4. *B. licheniformis* which causes abortion in cattle & sheep.
5. *B. thuringiensis* which consider insect pathogens.

INTRODUCTION

- Ubiquitous, present in Soil, Air, Dust, & Water.
- Frequently isolated as “**LAB CONTAMINANTS**”.
- *B. anthracis*, the causative agent of an important **Zoonotic disease called “ANTHRX”**.
- *B. cereus* can cause “**FOOD POISONING**”.
- All members are generally “**MOTILE**” except *B. anthracis*, which is “**NON-MOTILE**”.
- Temp. range for growth 25 -75°C.
- Salt conc. 2% - 25%.
- **Also gained importance recently because of its ability to be used as “Biological Weapon”**.

MORPHOLOGY

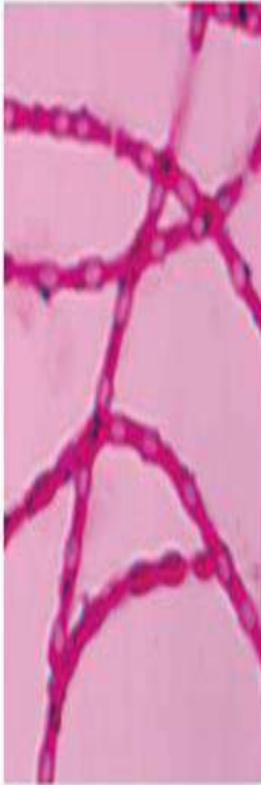
- The bacilli are arranged end to end in long chains.
- The ends are often concave & somewhat swollen so that a chain of bacilli present a "*bamboo-stick*" appearance.
- **Spore are oval & central in position & are of the same width as the bacillary body so that they do not cause bulging of vegetative cell.**
- Spores are formed in culture and in soil but never in host body.



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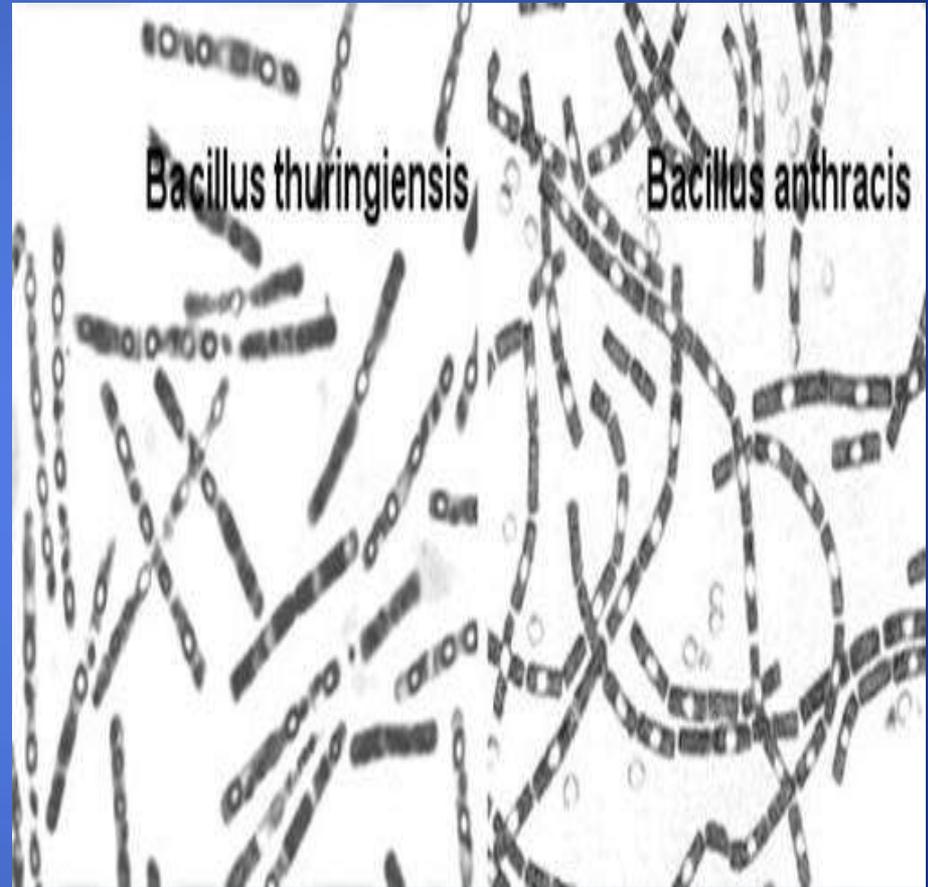
(a) *Bacillus anthracis*

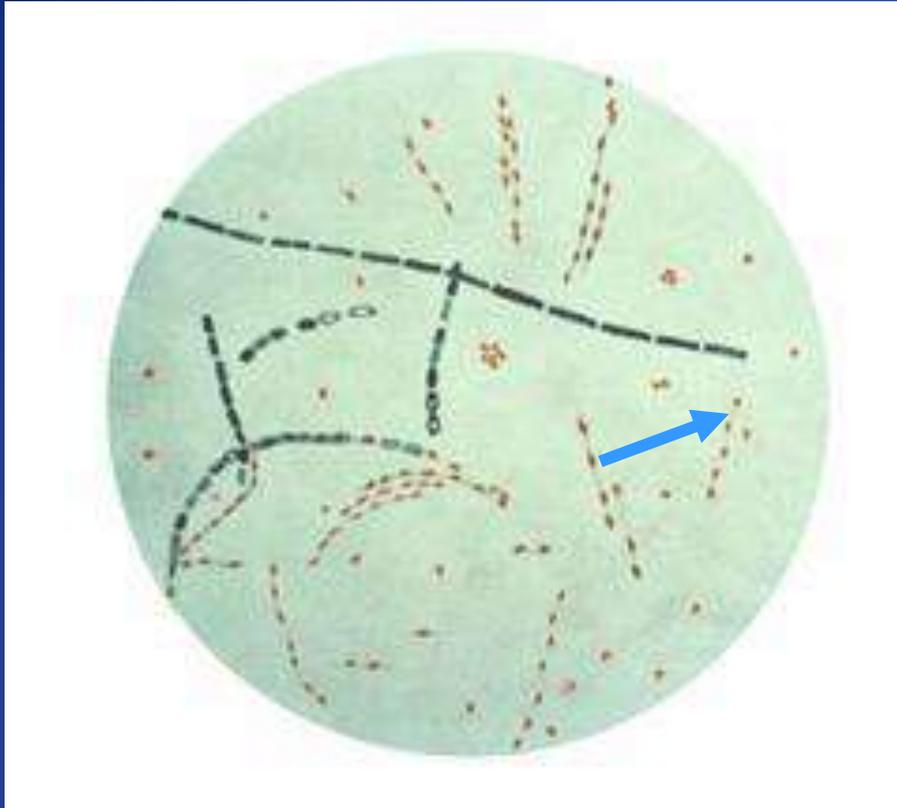


(b) *B. subtilis*

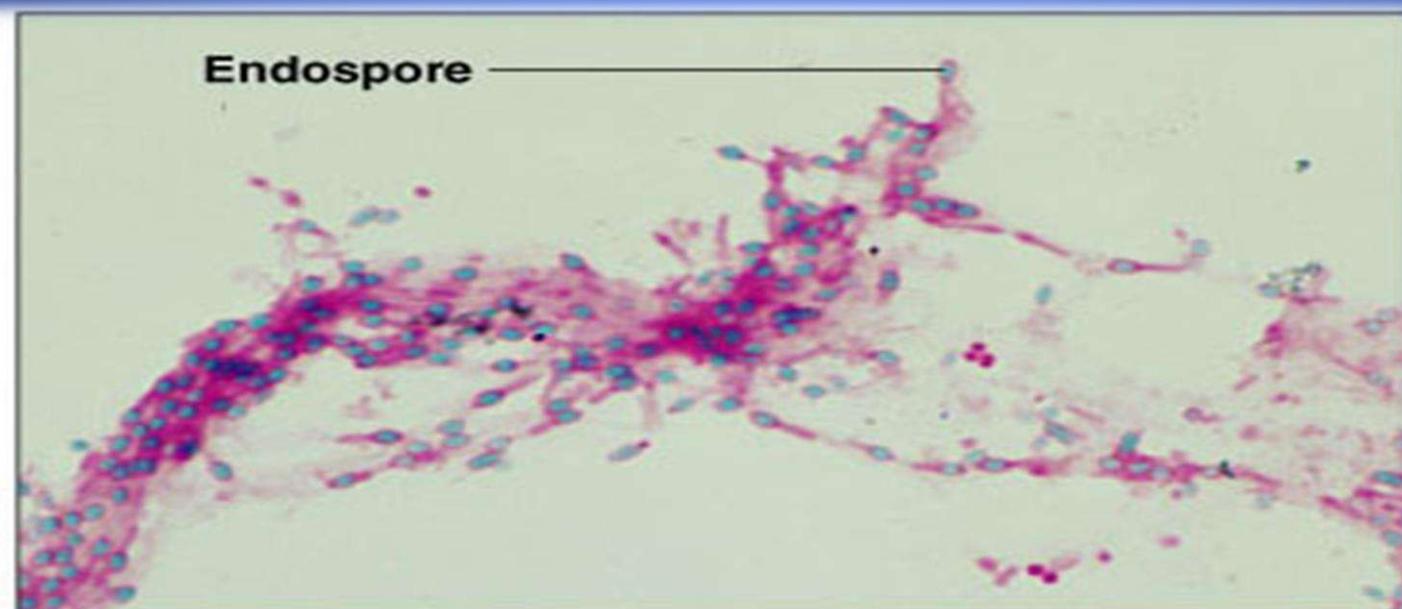
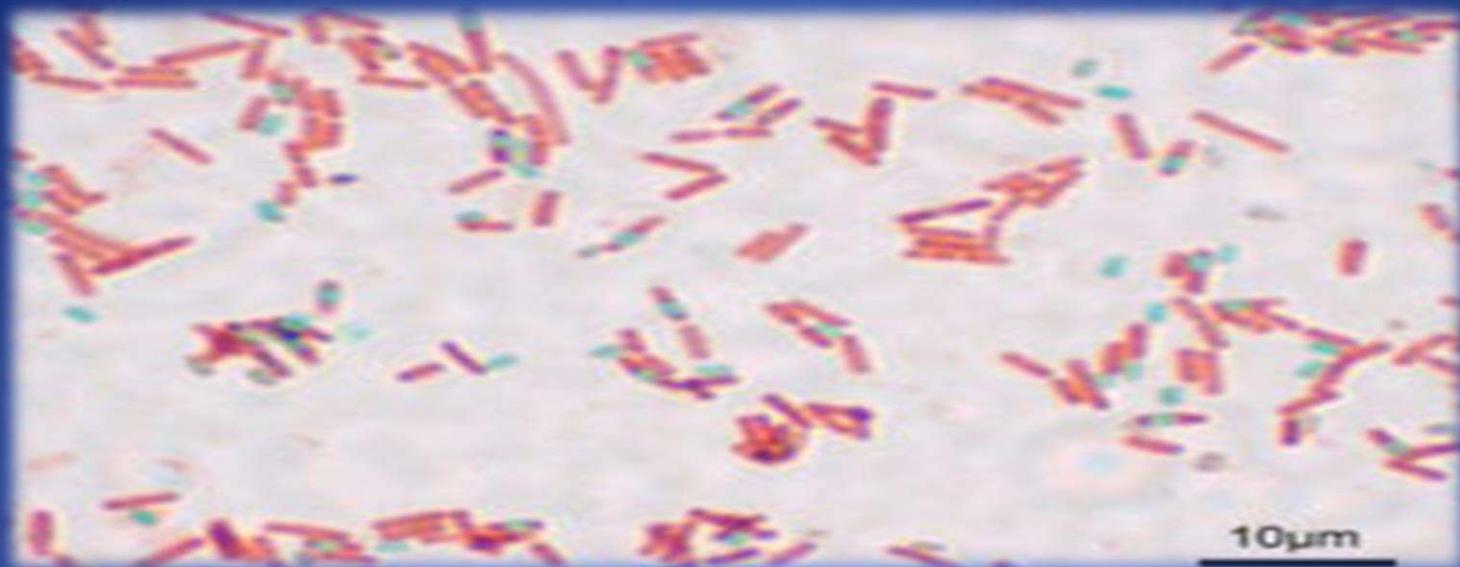


(c) *B. cereus*

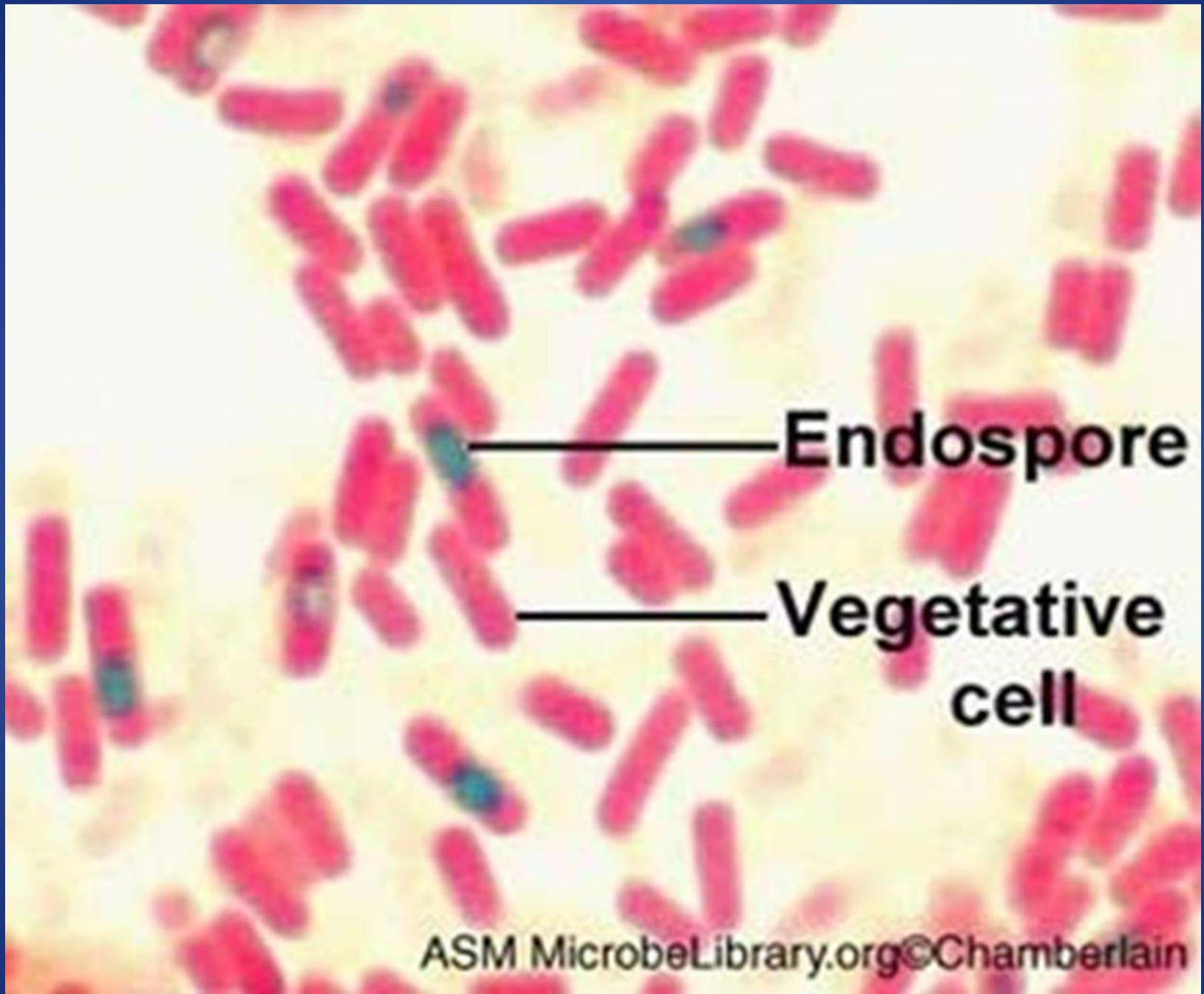




Photomicrograph of *Bacillus anthracis* (fuchsin- methylene blue spore stain).



(b) Endospore staining.

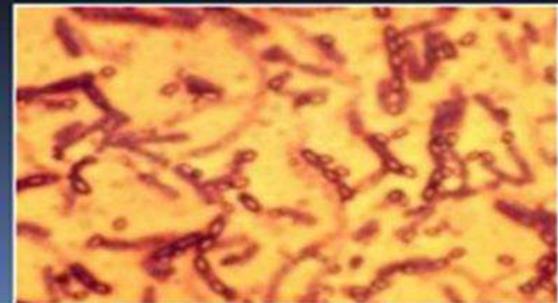


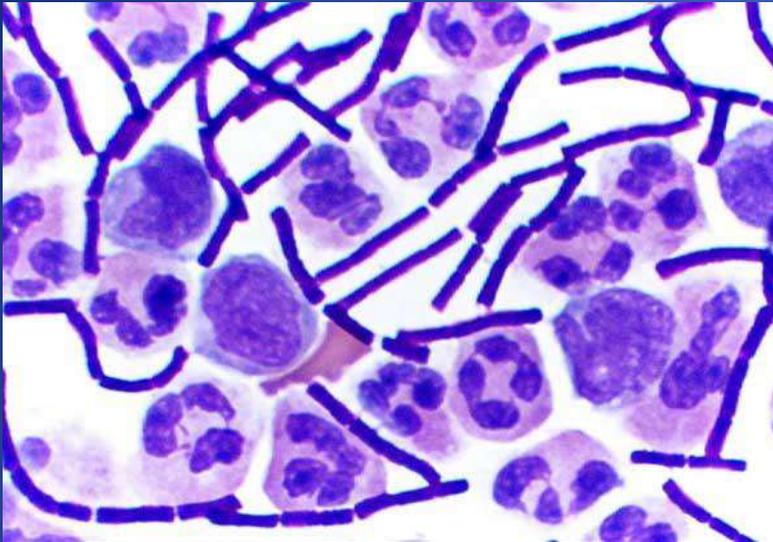
Endospore

**Vegetative
cell**

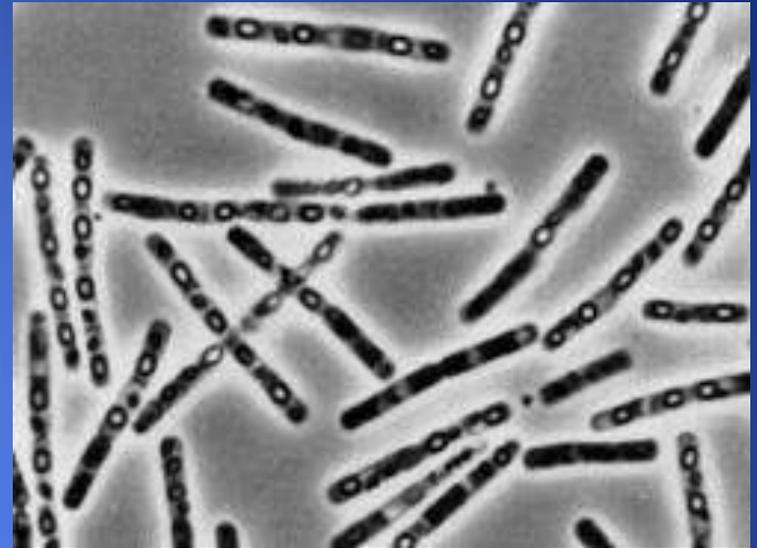
MICROSCOPY

- Gram stained smear from the specimen shows often chain of large Gram Positive Bacilli.
- Capsule appears as a clear halo around the bacterium by India-Ink preparation/ staining.
- Capsules are produced in the presence of bicarbonates or 10-25% CO₂
- Spores are oval and centrally located, non bulging
- Spores are stained by special stains – Sudan black B.



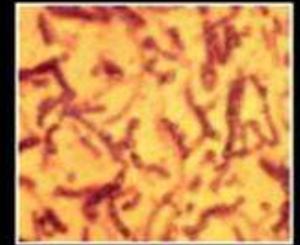


- ***Bacillus cereus*** .Gram stain.
450X. Bacilli are large bacteria, so that they are readily observed with the microscope's "high dry objective"but you can't detect anything about their spores. This could be a *Lactobacillus* .



- ***Bacillus thuringiensis***.
Phase Photomicrograph of vegetative cells, intracellular spores (light) and parasporal crystals (dark). 1000X.

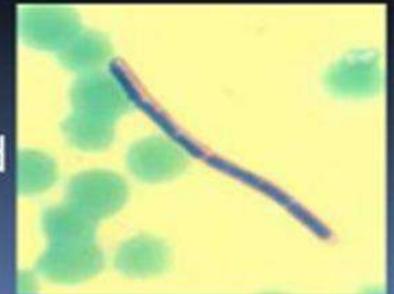
MORPHOLOGY



- Gram positive, Non-Acid fast, **Non-Motile.**
- Large (3-10 μm X 1-1.6 μm), rectangular
- Capsule is made up of **polypeptide, polymer of d-glutamic acid.**
- **This capsule is Plasmid coded (pXo2).**
- **It inhibits complement mediated phagocytosis.**
- Capsules not formed under ordinary conditions only if media containing bicarbonate or are incubated at 10 to 25 % CO_2 .
- If media contains serum, albumin, charcoal or starch – Capsule formation may occur in absence of CO_2 .

Spores

- Spores are stain by special methods – Sudan black – B – Fat globules maybe made out within bacilli. “Hot malachite green (Ashby’s method) OR 0.25% Sulphuric acid as spores are Acid fast”.
- When blood films are stained with polychrome methylene blue for a few seconds and observed – an amorphous purplish material is noticed around the bacilli.
- This represents the capsular material and is characteristic of anthrax bacillus.
- This is called as **“Mc Fadyean’s reaction”** & used as presumptive diagnosis of Anthrax in animals.



**B. anthracis in McFadyean
Reaction**

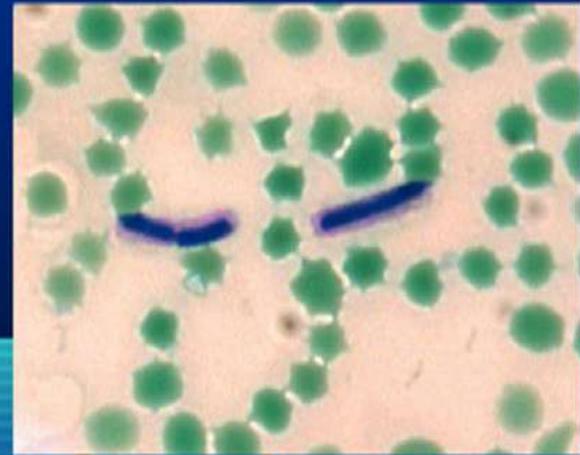


B. anthracis in Spore stain

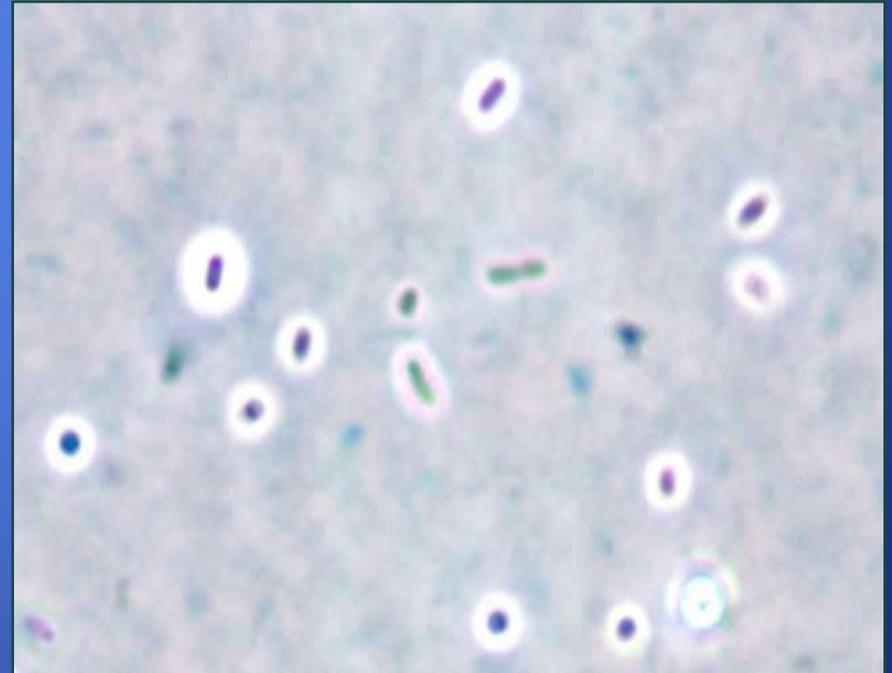
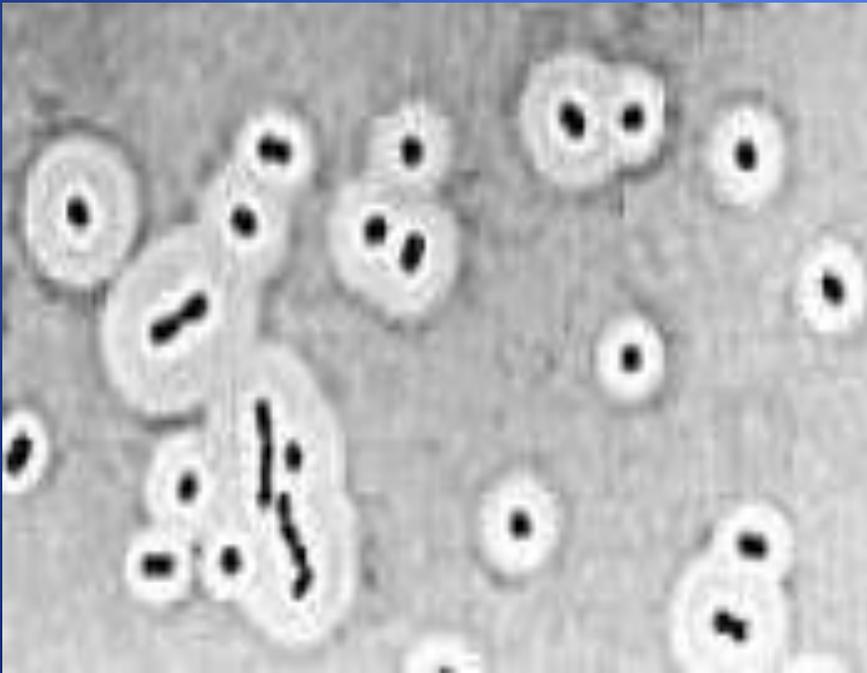


Produce Capsule in vivo by using **Giemsa** or can be seen in (**polychrome Methylene blue**) stained smears for 10-12 seconds & examined under microscope reveal square-ended blue rods in short chains with amorphous pink capsule material is noticed around the bacilli this is called (Mc' Faydean reaction) , (that disintegrated capsular material), This test is employed for presumptive diagnosis of anthrax.

Bacillus anthracis McFaydean capsule stain, grown at 35°C, in defibrinated horse blood.



Acidic dyes eg. India ink or Sudan black gives a semi opaque background against the clear capsules can be easily visualized. Capsules appear as clear zones (halos) around the retractile organism.



CULTURAL CHARACTERISTICS

- It is an **aerobe**, and **facultative anaerobe**, with a temperature range for growth of **12-45 °C** (optimum 35-37 °C).
- Good growth occurs on **ordinary media**. On agar plates, irregularly round colonies are formed, 2-3 mm in diameter, raised, dull, opaque, greyish white, with a frosted glass appearance.
- Under the low power microscope, the edge of the colony is composed of long, interlacing chains of bacilli, resembling locks of **matted hair**. This is called the '**Medusa head appearance**'.



Medusa head appearance

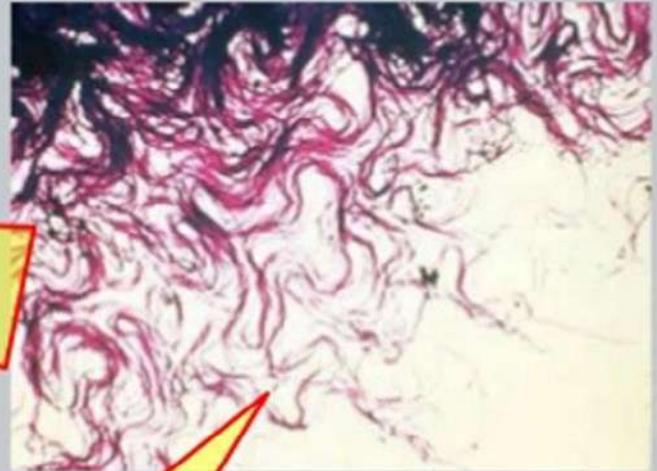


Fig. 2 : Gram positive bacilli in chains with spores (black arrow) and capsular halo (red arrow)

1. *B. anthracis* on nutrient agar:

growth in this agar containing 0.7% sodium bicarbonate in presence of 5-10% CO₂, the colonies are gray- white opaque mucoid colony due to capsule production with irregular margins from which comma- shaped out growth So called Medusa head formation.



2. *B. anthracis* on sheep blood agar :

grow well within 18- 24 hours , non haemolytic, colonies are large, opaque, flat, dry with irregular edge, white- gray to green in colour with granular ground-glass appearance



BACILLUS CEREU

- B cereus has become an **important cause of food poisoning**.
- It is widely distributed in nature and may be readily isolated from soil, vegetables and a wide variety of foods including milk, cereals, spices, meat and poultry.
- B cereus is generally motile but nonmotile strains may occur.
- **It resembles B anthracis** except that it is not capsulated and does not react with anthrax fluorescent antibody conjugate.
- B cereus produces two patterns of **foodborne disease**.
- One is associated with a wide range of foods including **cooked meat and vegetables**.

2. *B. cereus*: On sheep blood agar:

the morphology resembles that of *B. anthracis* but they tend to be slightly larger, green in colour. most strains are surrounded by wide zone of complete haemolysis & give strong Lecithinase activity on egg Yolk agar after 24 hours.



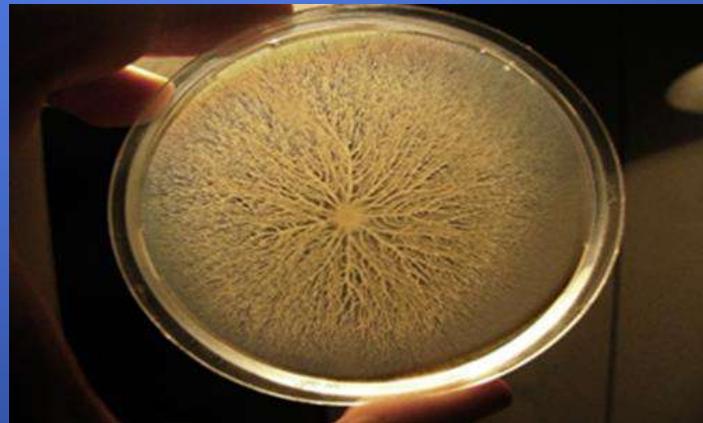
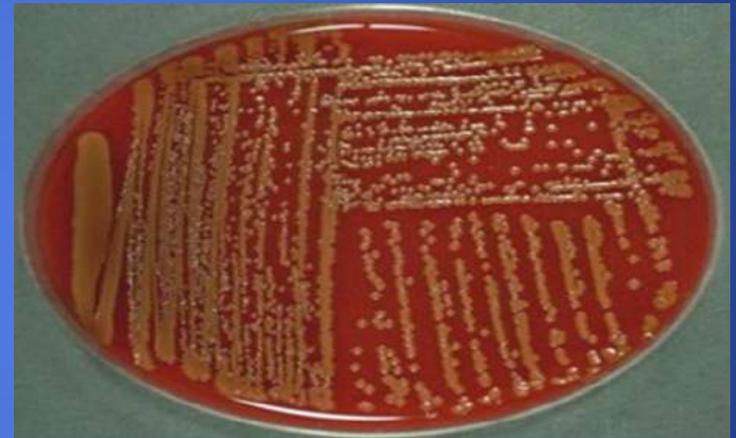
3. *B. mycoides*: On nutrient agar

has Rhizoid type colonies like fungal appearance after incubated at 25- 30 oC for 3 days.



4. *B. subtilis*: On sheep blood agar

showing round to irregular colonies with a dull, Wrinkled, granular. Cream to brown surface. some strains are haemolysis, don't bulge the spore.



5. *B. licheniformis*: On sheep blood agar

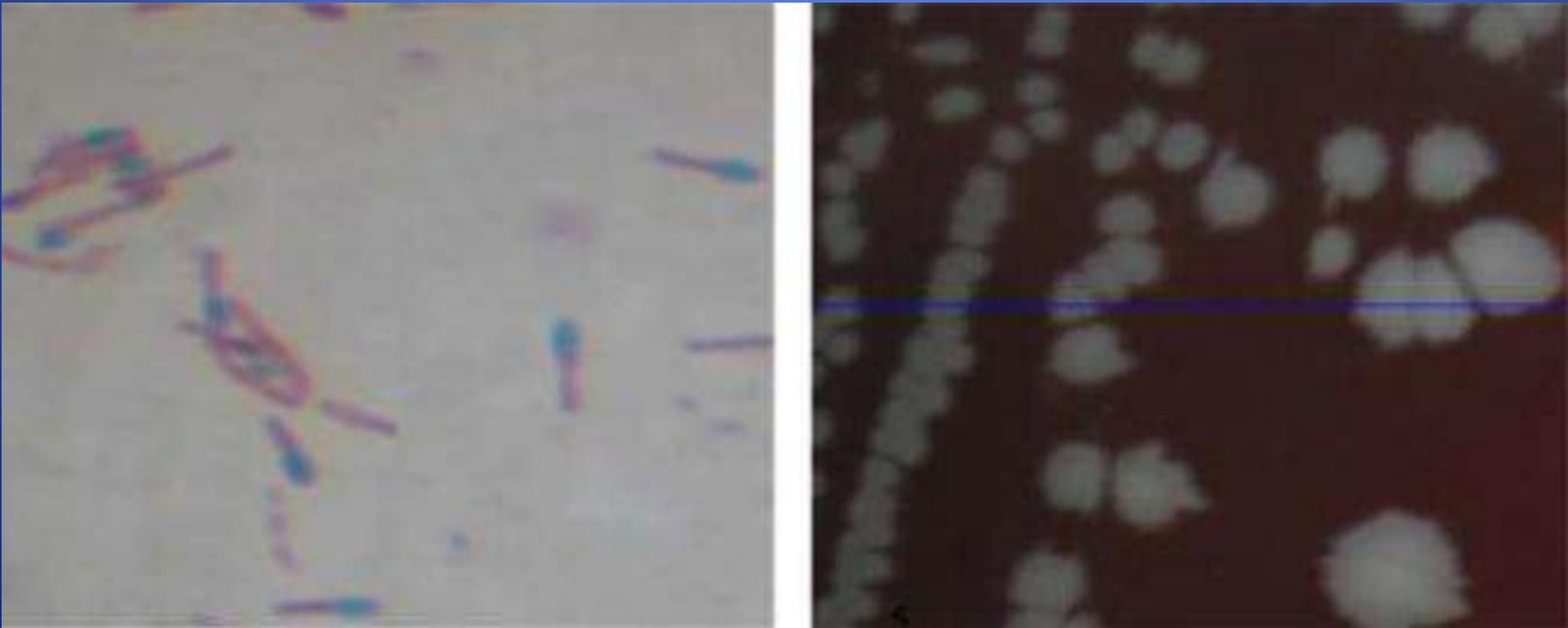
inoculated as a streak to show the Heaped wrinkled lichen-like appearance. The colonies are opaque, dull, rough & unreactive on egg yolk agar



6. *B. circulans*:

are motile & moving outwards over the surface of agar in a circular manner. The plate incubation at 25°C for 3 days.

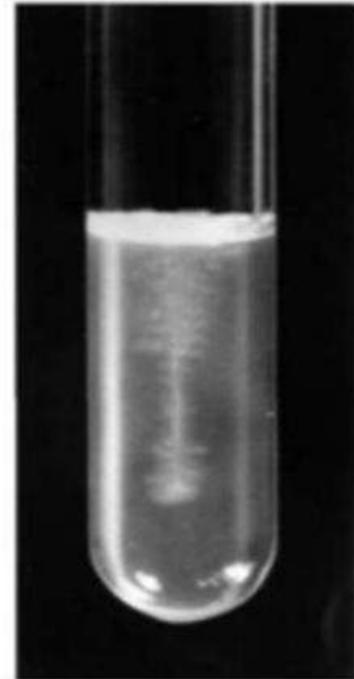
B. circulans as Gram variable & the cells are often Gram – negative even in smears from young cultures

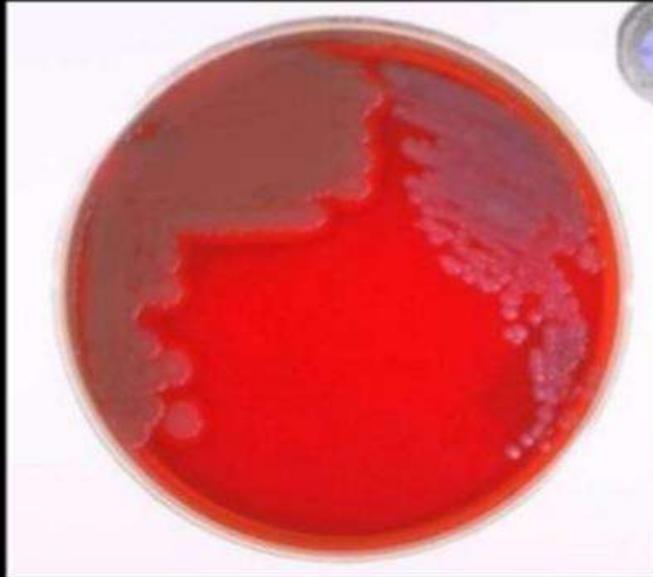


BIOCHEMICAL REACTIONS

- Glucose, maltose and sucrose are **fermented** producing acid but no gas.
- Nitrates are reduced to nitrites.
- Catalase is formed.
- On gelatin stab culture, a characteristic 'inverted fir tree' appearance is seen, with slow liquefaction commencing from the top.

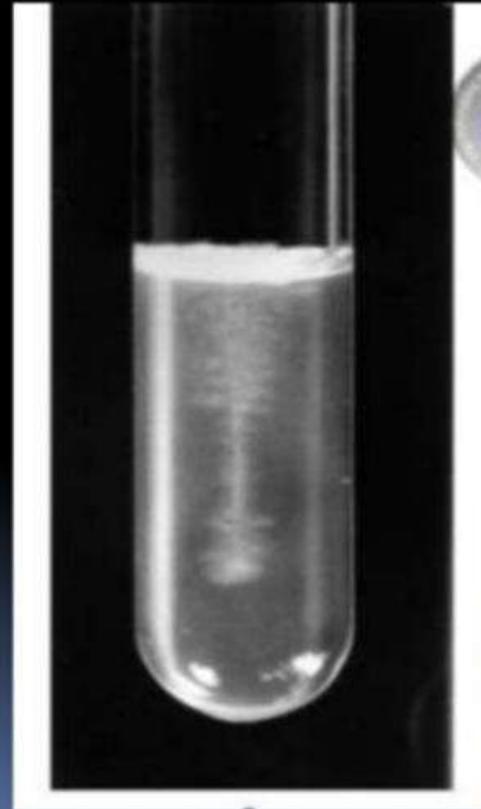
Gelatine stab culture





Medusa Head Appearance
-wavy colonies with small
projections

Inverted fir tree



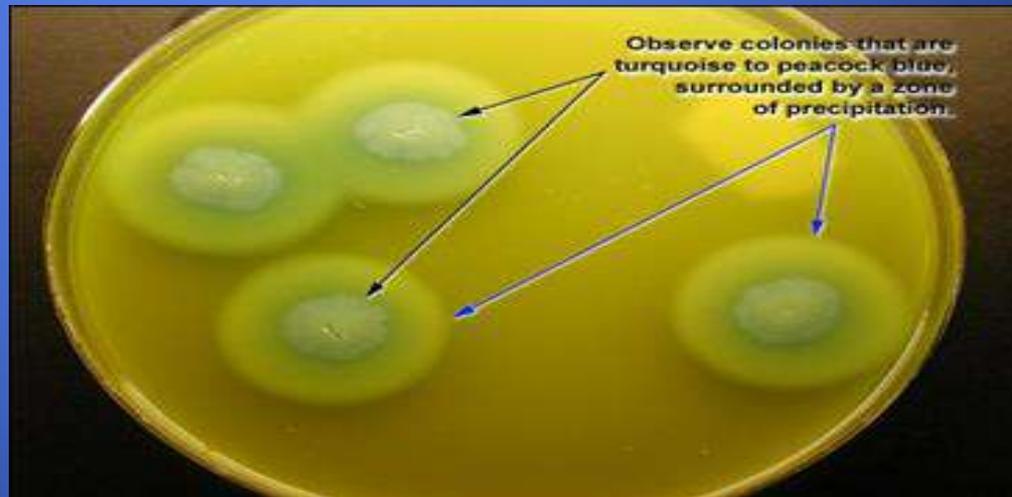
1. Selective medium for *B. anthracis*: (PLET)

consisting of heart infusion agar with polymyxin - lysozyme, Ethylene Diamine tetra acetic acid (EDTA) & thallos acetate has been devised for isolation of *B. anthracis* from mixtures containing other spore-bearing bacilli.

2. special MYPA medium :

Mannitol- egg yolk- phenol red- polymyxin agar

B. anthracis gives weak Lecithinase activity opaque zone after 48 hours.



Selective medium:(PLET medium)

- Consisting of heart infusion agar with polymyxin, lysozyme, ethylene diamine tetracetic acid and thallos acetate.
- It is used for the isolation of *B. anthracis*.



PLET MEDIUM showing growth of Anthrax bacilli

3. Penicillin agar medium:

Solid medium containing 0.05-0.5 units of penicillin /ml in 3-6 hrs. The cells become large, spherical because of their weaker cell walls under the action of penicillin. and occur in chains on agar surface, resembling a (STRING OF PEARLS), *B. cereus* similar to *B. anthracis* tend to slightly, larger, have a slightly greenish hue and most strain surrounded by a wide zone of hemolysis.



- “String of Pearls reaction” – solid medium containing 0.05-0.5 units of Penicillin/ml, in 3-6 hrs. the cells become large, spherical and occur in chains on agar surface, resembling a string of pearls.
- Selective medium – PLET medium – contains Polymyxin, Lysozyme, EDTA & Thallous acetate : to isolate it from mixtures containing other spore bearing bacilli.



Test	B.anthraxis	B. cereus	B. mycoides	B. thuringiensis
Motility	-	+	-	+
Heamolysis	- or weak	+	weak	+
Pencillin susceptibility	S	R	R	R
Gelatin stab culture	invert fir tree	rapid liquefication	rapid liquefication	rapid liquefication
Lecithinase activity (egg-yolk agar)	+weak	+	+	+
Nutrient agar with 0.7% Na bicarbonate under 10%CO2	mucoid colony	unchange	unchange	unchange
Pathogenesis mice or guinea pigs	+(death in 24-48 hour)	+ large doses	-	-