Practical No.14

Genus Bacillus

G+ve aerobic spore-forming bacilli

Two *Bacillus* species are considered medically significant: *B. anthracis*, which causes anthrax, and *B. cereus*, which causes a foodborne illness. A third species is *B. subtilis*, an important model organism. It is also a notable food spoiler; it is also one of the contaminants of culture media in bacteriology laboratories.

Bacillus anthracis

Bacillus anthracis is a Gram-positive spore-forming, rod-shaped bacterium**.** *Bacillus anthracis* spores in particular are highly resilient, surviving extremes of temperature, low-nutrient environments, and harsh chemical treatment over decades or centuries. It is the causative agent of anthrax**.** Three types of anthrax; 1. Cutaneous anthrax (malignant pustule). 2. Pulmonary anthrax (wool sorter's disease) and 3. Gastrointestinal anthrax.

Laboratory diagnostic tests;

Specimen; pus, sputum, stool.

Smear; stained with gram's stain. Appears as large G +ve bacilli arranged singly, pairs or in chains with squared ends. In laboratory media, the bacilli form long chains. Spores develop at the end of the log phase of multiplication. These are central / subterminal, ellipsoidal and non-bulging and resist Gram stain, appearing as unstained areas within the cell. With malachite green/safranine (or malachite green/ basic fuchsin) staining, the spores are stained green and the vegetative forms are pink. In the Ziehl-Neelsen staining, spores are pink and the vegetative forms are blue. Capsules are lost in normal culture, the capsule is only seen if the specimen is taken from animal's exudates or from blood**,** by using a Mcfadyean reaction, capsule appears reddish purple and the bacillus appear blue. Mcfadyean reaction involves staining with polychrome methylene blue.

Culture; on blood agar and under aerobic conditions colonies appear white, granular, circular disks which have margins resembling a lock of hairs (the medusa head).

Biochemical reactions; gelatin liquification ; *Bacillus anthracis* gives a positive result.

Differential characteristics of *B. anthracis* and non-pathogenic *B.* species

No.	Characters	*B. anthracis*	Non-pathogenic *Bacillus spp.*
1.	Capsule	Present (in clinical specimens)	Absent
2.	Motility	Negative	Positive
3.	Haemolysis on Blood Agar	Non-haemolytic	ß haemolytic
4.	Gelatin liquefaction	Slow	Rapid
5.	Susceptibility to penicillin	Susceptible	Resistant
6.	Animal pathogenicity	Pathogenic (causes anthrax)	Generally non-pathogenic

Serological tests;

Ascoli precipitin test; is used for recognition of anthrax infection. About 2 grams of tissue is boiled for 5 minutes with 5 ml normal saline to which acetic acid has been added. The fluid is filtered, 0.5 ml of anthrax antiserum is placed in a narrow tube and clear filtrate is carefully layered on the top of the serum. The development of a white ring of precipitate at the junction of the two fluids within 10 minutes at room temperature, denote positive results.