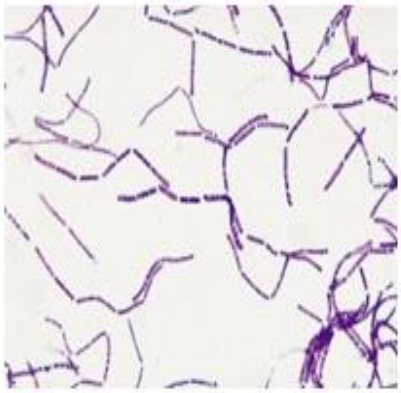
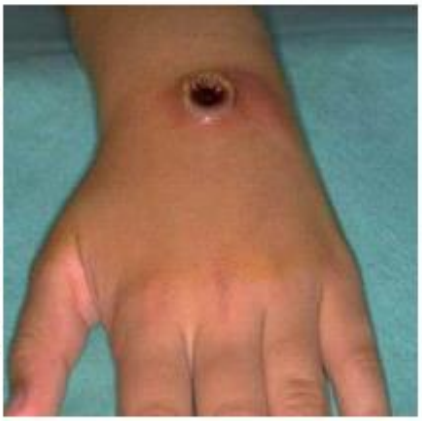
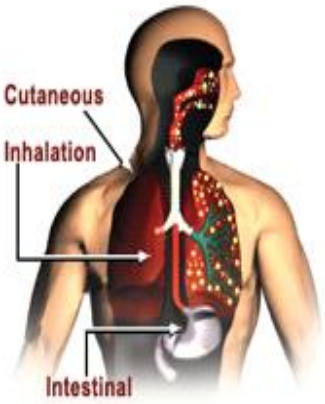
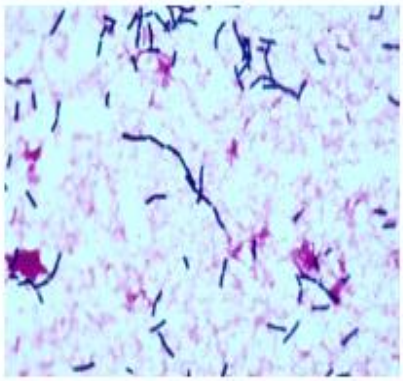






Introduction:

The genus Bacillus currently comprises in excess of 60 species, commonly found in the environment and as laboratory contaminants The aerobic endospore-forming bacteria can be recovered from almost every from dry Antarctic valleys , thermal sites, marine and other aquatic sites and they comprise a major proportion of the soil microflora.
Some types of Bacillus bacteria are harmful to humans, plants, or other organisms.

Their collective features include degradation of most all substrates derived from plant and animal sources, including cellulose, starch, pectin, proteins, agar, hydrocarbons, and others; antibiotic production; nitrification; denitrification and nitrogen fixation.

Characteristics:

- Gram-positive, rod shaped cells.
- *Bacillus* cells are typically fairly rectangular rods, often occuring in pairs or chains, mostly straight, 0.3-2.2 x 1.2-7 μm
- Endospore-forming (outside the host).
- Capsulated (inside the host)
- Mostly saprobic
- Aerobic or facultatively anaerobic
- Catalase positive
- Versatile in degrading complex macromolecules
- Primary habitat is soil.

- Many bacillus species are haemolytic, a useful characteristic in differentiating them from B. anthracis(which is non-haemolytic).
- Motile , by peritrichous flagella, a notable exception is Bacillus anthracis).
- Most species are oxidase-positive, which may lead to confusion with Pseudomonas species, especially if the Bacillus species are poorly stained.
- 3 species of medical importance:
    - ***Bacillus subtilis***
    - ***Bacillus anthracis***
    - ***Bacillus cereus***

Bacillus species can be broadly divided in three groups based on the morphology of the spore and sporangium 3 . The groups are:

Group 1 - Gram-positive, produce central or terminal ellipsoidal or cylindrical spores that do not distend the sporangium.

Group 2 - Gram-variable with ellipsoidal spores and swollen sporangia.

Group 3 - Gram-variable, sporangia swollen with terminal or subterminal spores.

Specimen:

(B. anthracis): Sputum; Blood; Vesicle fluid; Stool; Cerebrospinal fluid (CSF). (Bacillus cereus): soil, on vegetables, and in many raw and processed foods.

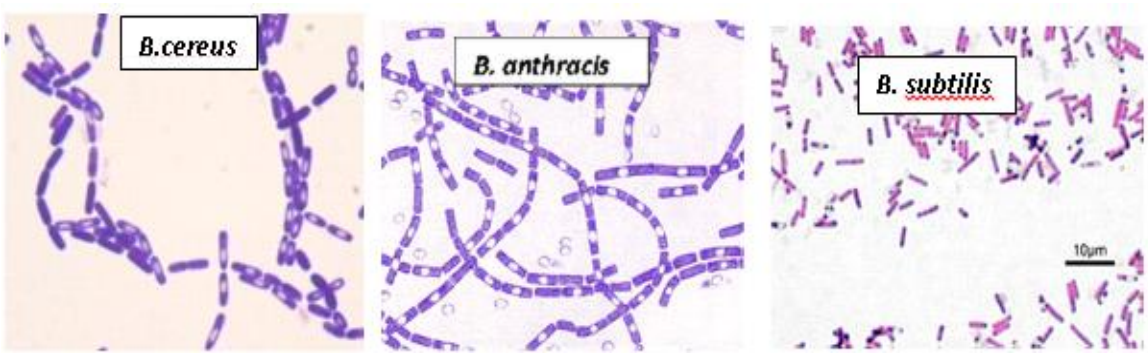
Pathogenicity:

Most strains of B*acillus* are not pathogenic for humans but may, as soil organisms, infect humans incidentally.

- ***B***. ***cereus*** causes spoilage in canned foods .and **food poisoning** of short duration.
- ***B. subtilis*** is a common contaminant of laboratory cultures and is often found on human skinA
- ***B***. ***anthracis***, causes anthrax in humans and domestic animals.
- **B. *thuringensis*** produces a toxin (Bt toxin) that causes disease in insects , used for biocontrol purposes.

Lab work:

1. Gram stain:



Gram positive sporulated bacilli of genus Bacillus

2. Spore stain (observe shape and position of spore):

In the Schaeffer-Fulton`s method, a primary stain-malachite green is forced into the spore by steaming the bacterial emulsion. Malachite green is water soluble and has a low affinity for cellular material, so vegetative cells may be decolourized with water. Safranin is then applied to counterstain any cells which have been decolorized. At the end of the staining process, vegetative cells will be pink, and endospores will be dark green.

Reagents used for Endospore Staining

Primary Stain: Malachite green (0.5% (wt/vol) aqueous solution)
0.5 gm of malachite green
100 ml of distilled water

Decolorizing agent
Tap water or Distilled Water

Counter Stain: Safranin
Stock solution (2.5% (wt/vol) alcoholic solution)
2.5 gm of safranin O
100 ml of 95% ethanol

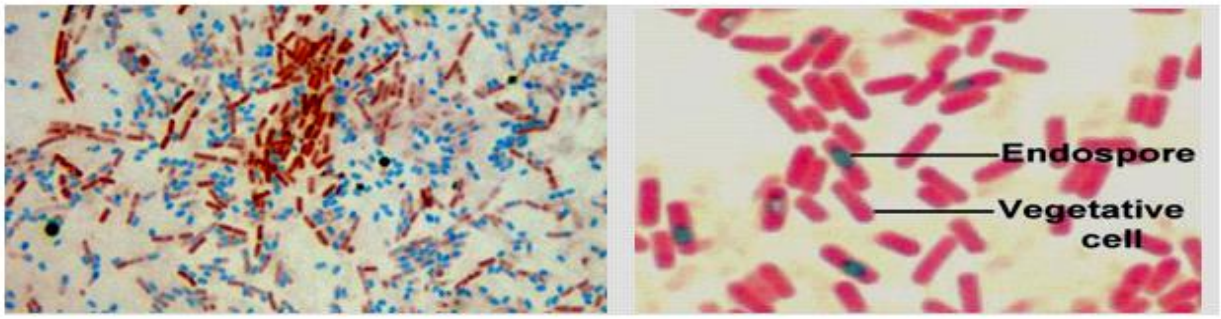
Schaeffer-Fulton method for staining endospores

- Air dry and heat fix the 3-7 days old culture on a glass slide .

Note!!!!!!! Older cultures should be used when performing spore stain because they are lacking in nutrients and in competitive living environment.

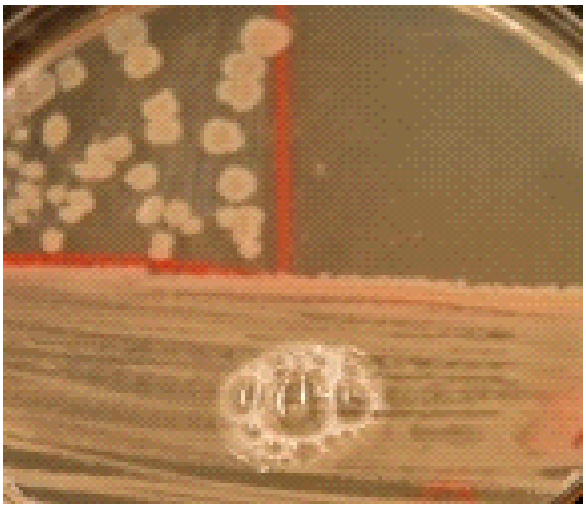
- Saturate with malachite green stain solution and steam for 5 minutes, keeping the slide moist and adding more dye as required. Alternatively, the slide may be steamed over a container of boiling water.
- Wash the slide in tap water.
- Counterstain with safranin for 30 seconds. Wash with tap water; blot dry.

- Examine the slide under the oil immersion lens (1,000X) for the presence of endospores. Endospores are bright green and vegetative cells are brownish red to pink.



Schaeffer-Fulton method for staining endospores

3. Catalase test (3% H_2O_2 reagent) :



Catalase : positive

4. Motility (on semisolid agar or hanging drop slide):

There are a variety of ways to determine motility of a bacterium—biochemical tests as well as microscopic analysis. If fresh culture of bacteria is available, microscopy is the most accurate way to determine bacterial motility and '**Hanging drop method'** is a commonly used microscopic technique.

Semisolid agar:

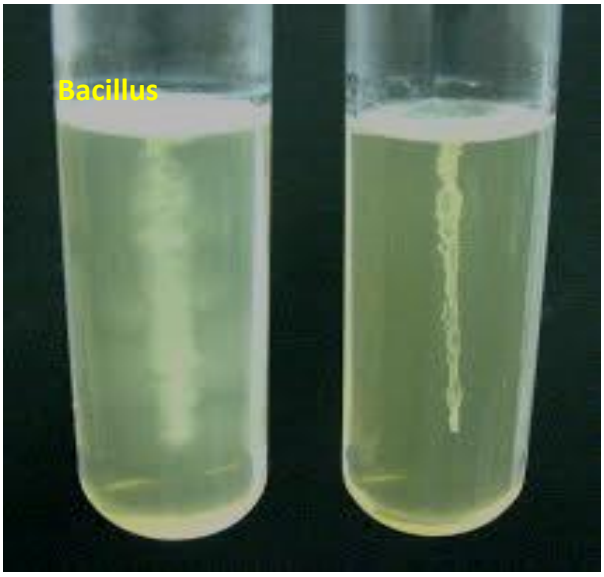
Procedure:

- Prepare a semisolid agar medium in a test tube.
- Inoculate with a straight wire, making a single stab down the center of the tube to about half the depth of the medium.
- Incubate under the conditions favoring motility.

- Incubate at 37°C
- Examine at intervals, e.g. after 6 h, and 1 and 2 days *(depends on generation time of bacteria) . Freshly prepared medium containing 1% glucose can be used for motility tests on anaerobes.*

Results: Hold the tube up to the light and look at the stab line to determine motility.

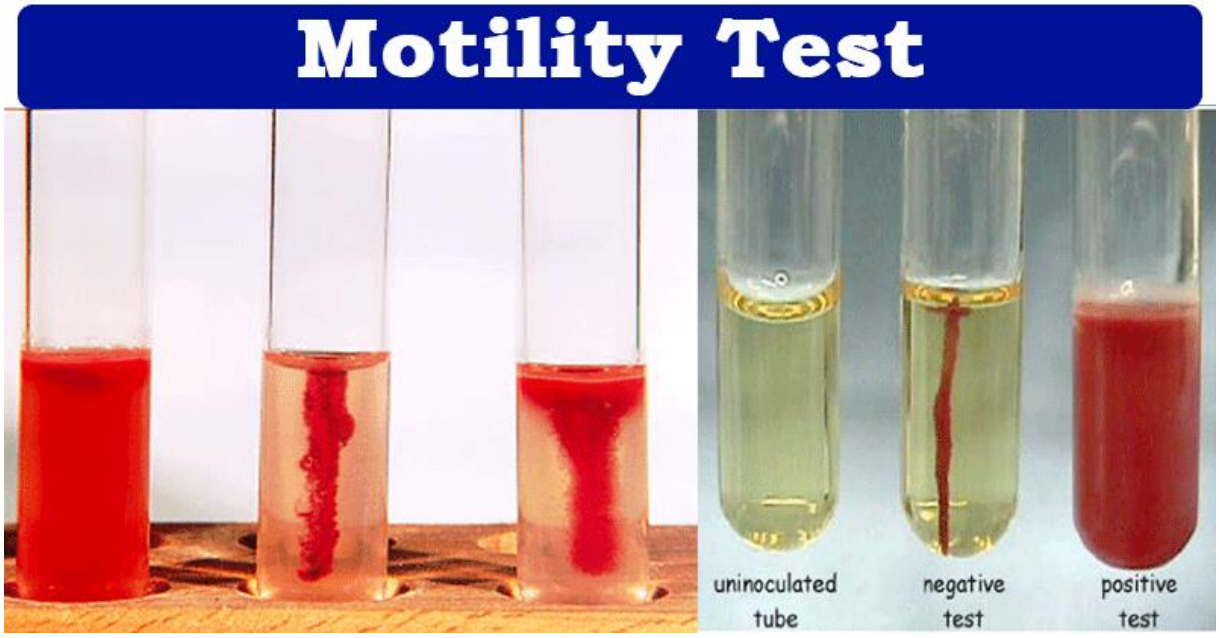
- **Non-motile bacteria** generally give growths that are confined to the stab-line, have sharply defined margins and leave the surrounding medium clearly transparent.
- **Motile Bacteria** typically give diffuse, hazy growths that spread throughout the medium rendering it slightly opaque.



Motility : positive

Note:

Incorporation of tetrazolium chloride at a final concentration of 0.005% in the medium is helpful. The tetrazolium makes the motility agar much **easier to read** for motility. Tetrazolium is colourless in oxidized form, but the **reduced salt is red** (occurring as a result of bacterial metabolism) and indicates where bacterial growth has occurred.



5. Starch hydrolysis: (Starch agar and Gram's iodine reagent).

A qualitative test used to identify bacteria that can hydrolyze starch (amylose and amylopectin) using the enzymes a-amylase and oligo-1,6-glucosidase. Some bacteria have the gene for making the enzyme amylase and can break down starch to sugar. This test is often used to differentiate species from the genera Clostridium and Bacillus. Because of the large size of amylose and amylopectin molecules, these organisms cannot pass them through the bacterial cell wall. In order to use these starches as a carbon source, bacteria must secrete a-amylase and oligo-1,6-glucosidase into the extracellular space. These enzymes break the starch molecules into smaller glucose subunits which can then enter directly into the glycolytic pathway of bacterial metabolism.

Starch agar is a simple nutritive medium with starch added. Since no color change occurs in the medium when organisms hydrolyze starch, we add iodine to the plate after incubation. Iodine turns blue, purple, or black (depending on the concentration of iodine) in the presence of starch. A clearing around the bacterial growth indicates that the organism has hydrolyzed starch.

Starch hydrolysis medium:

Composition: Ingredients gms / litre

Beef extract 3.000

Starch, soluble 10.000

Agar 12.000

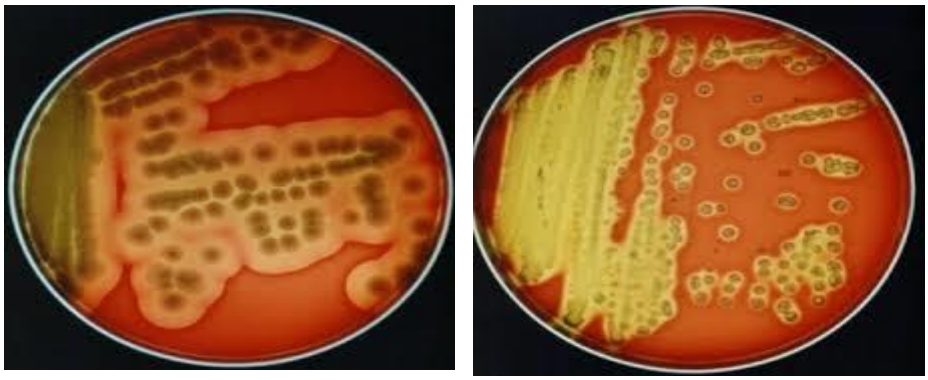
Final pH (at 25°C) 7.5±0.2

Directions: Suspend 25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates

Positive starch hyrolysis test

6. Nutrient agar and blood agar (observe colony morphology and hemolysis).

Bacillus colony morpholofy on nutrient agar.



Bacillus colonies on blood agar.