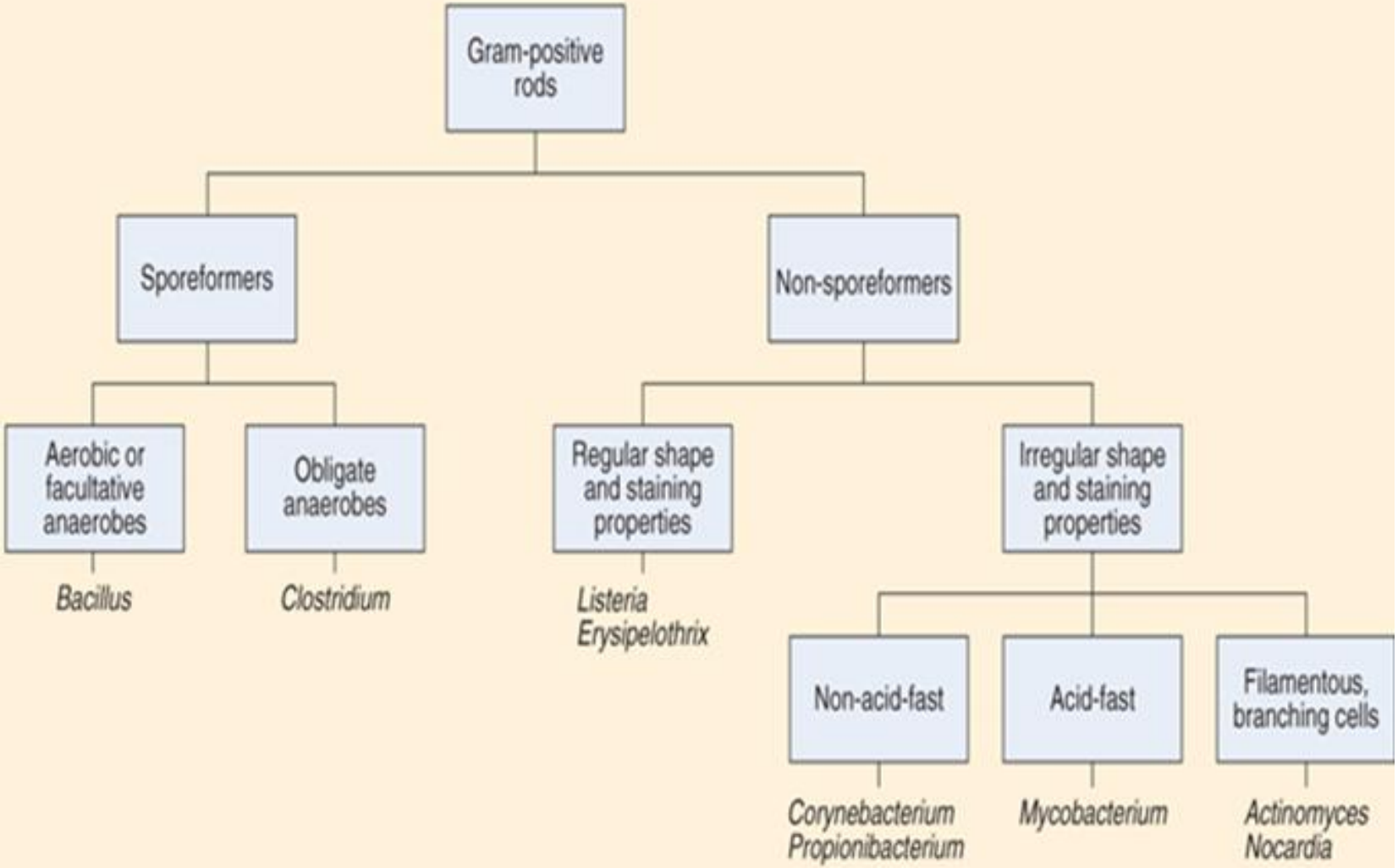
Practical
Medical Bacteriology

Lab 6

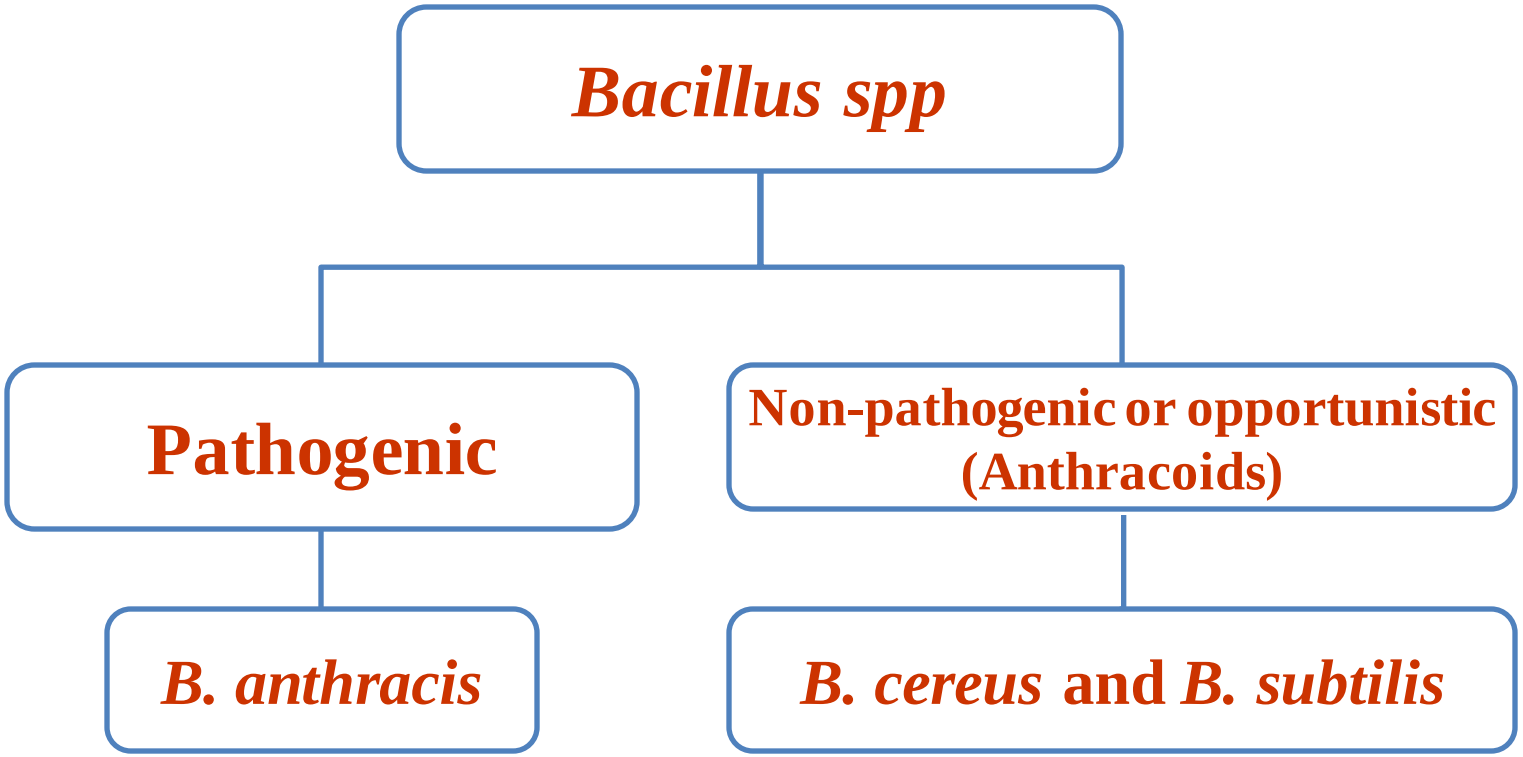
Laboratory Diagnosis of *Bacillus* spp

2021/2022

BLE 19.1 Scheme for Differentiating Gram-Positive Bacilli



Aerobic Spore Forming *Bacillus* spp



General Characters of *Bacillus* spp

- Large Gram positive spore forming bacilli.
- Most are saprophytic in soil, water, and air and called anthracoides such as *Bacillus cereus* and *Bacillus subtilis* .
- *Bacillus anthracis* as a major pathogen.
- All are motile except *B. anthracis*
- Aerobic or facultative anaerobic bacteria
- Catalase positive

Significant *Bacillus* spp

Bacillus anthracis

- large, non-motile, encapsulated, spore-forming Gram-positive bacilli
- Cause anthrax which infect herbivorous animals such as sheep.
- Humans acquire infection by contamination of wound or ingestion or inhalation of spores

Bacillus cereus

- Large, motile, saprophytic bacillus
- Non-capsulated
- A normal inhabitant of soil also isolated from food
- Causes food poisoning

Bacillus subtilis

- Common laboratory contaminant
- Tolerates very high temperatures

Bacillus stearothermophilus

- Used as indicator for efficacy of autoclave

Laboratory diagnosis

1. **Specimen:**
   - ***Bacillus anthracis***
   - Pastular exudates in malignant pustule (skin)
   - Sputum
   - Stool (stool specimen is emulsified and heating to 80 ˚C to kill non-spore forming microorganisms).

   - ***Bacillus cereus***
   - Normal stool flora, to diagnose food poisoning must culture suspected food NOT **stool**.

Laboratory diagnosis

2. Cultural characteristics

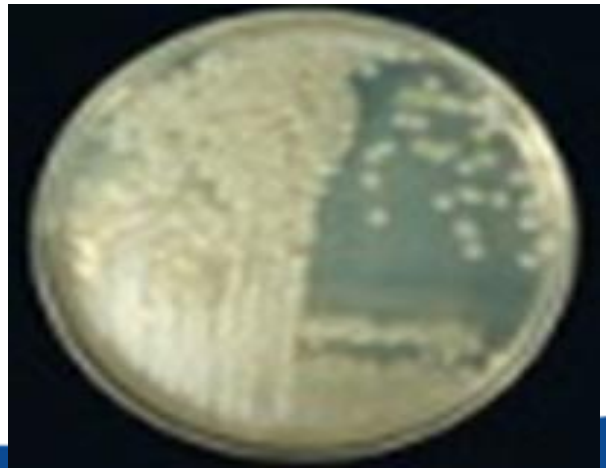
➢ On blood agar

– ***B. anthracis:*** Non-hemolytic, raised, large, grayish-white colonies with irregular, fingerlike edges described as beaten egg whites.

– ***B. cereus:*** β hemolytic; large, feathery, spreading colonies.

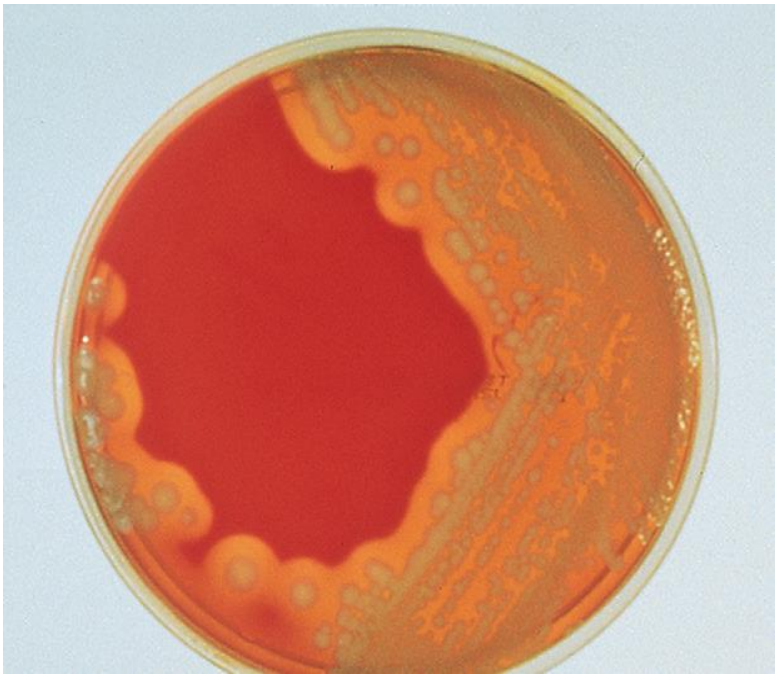
➢ On nutrient agar:

– ***Bacillus spp*** grow aerobically on nutrient agar at 37˚C with characteristic mucoid or smooth colonies, which indicates the pathogensity of organism (presence of capsule).



Laboratory diagnosis

***B. anthracis* on blood agar**



***B. cereus* on blood agar**

Laboratory diagnosis

3. Microscopical Morphology:

A. Gram stain:

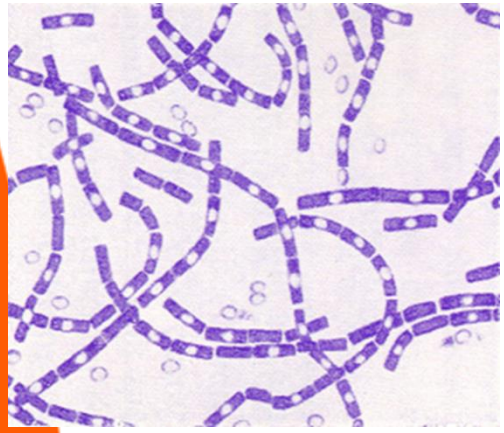
- ***Bacillus anthracis:*** large, square-ended Gram-positive rods to Gram-variable; may appear end to end giving a "**bamboo appearance**".
- ***Bacillus cereus:*** large, Gram-positive rods, can stain Gram-variable or Gram-negative.

B. Spore Stain:

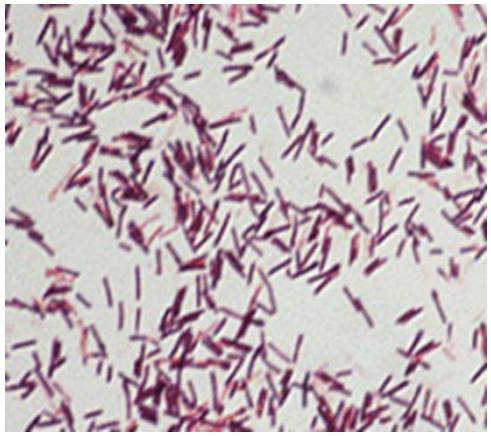
- ***Bacillus* spp spores** are oval, central and non-bulging
- By spore staining technique (Malachite green and safranine), the spore appears green while the vegetative cells appear red.

Laboratory diagnosis

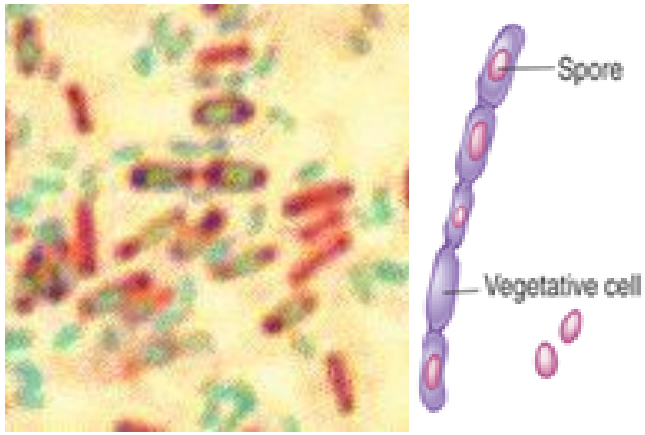
3. Microscopical Morphology:



Gram stain
B. anthracis



Gram stain
B. cereus

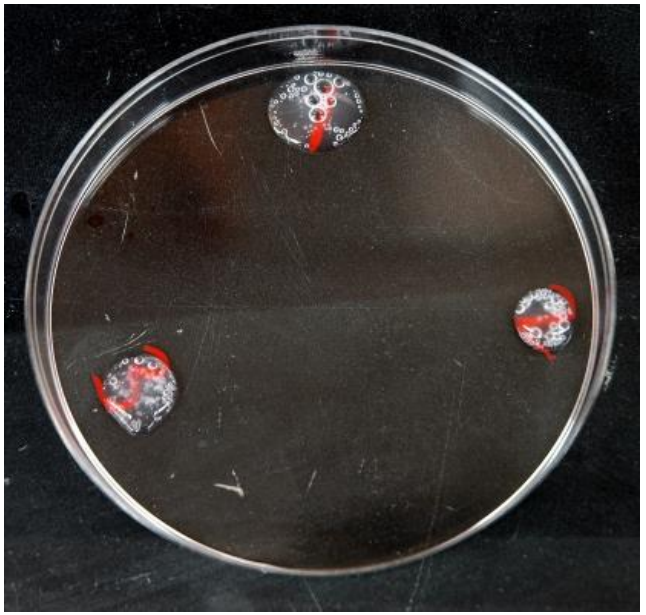


Spore stain

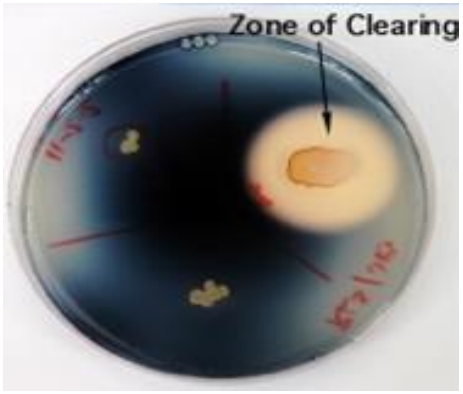
Laboratory diagnosis

4. Preliminary tests:

- **Catalase test:** all *Bacillus* species are catalase positive
- **Motility test:** all *Bacillus* species are motile except *B. anthracis*.



Laboratory diagnosis



5. Biochemical tests:

A. Starch Hydrolysis (Amylase Activity)

Principle:

- Starch + Iodine → blue color
- Glucose + Iodine → No reaction

Amylase iodine

Nutrient Agar containing Starch + M.O → Glucose → Appearance of colorless zone around the growth.

Procedure:

1. Inoculate nutrient agar plate containing 1% Starch with the M.O.
2. Incubate the plate at 37 ˚C for overnight.
3. After incubation, flood the plate with Iodine solution.

Results:

- Activity of amylase is indicated by a clear zone around the growth while the rest of the plate gives blue color after addition of iodine.

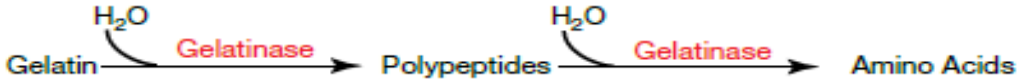
Laboratory diagnosis

5. Biochemical tests:

B. Gelatinase hydrolysis test:

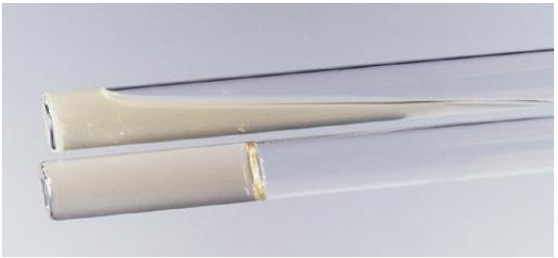
Principle:

Gelatin hydrolysis test is used to determine the ability of a microbe to produce gelatinase as extracellular enzyme that hydrolyze the gelatin.



Procedure:

1. Tubes of nutrient gelatin is stab-inoculating with microorganisms.
2. Incubate the tubes for 7 days at 37˚ C. 7-days incubation period is usually sufficient to see liquefaction of the medium.



Results:

1. Activity of gelatinase is indicating by a liquefaction medium
2. *B. cereus* is gelatinase-positive while *B. anthracis* is gelatinase-negative

Differential characteristics of *B. anthracis* & *B. cereus*

Tests	***B. anthracis***	***B. cereus***
Haemolysis on BA	-	+ (β)
Motility	Non motile	Motile
Starch hydrolysis	+	+
Gelatin hydrolysis	-	+
Penicillin susceptibility	S	R
API 20E system for identification of *Bacillus* species		