Bactria Classification

B. anthracis

Lab 3

Spore-Forming Gram Positive Bacilli

Scientific Classification of Bacillus Spp.

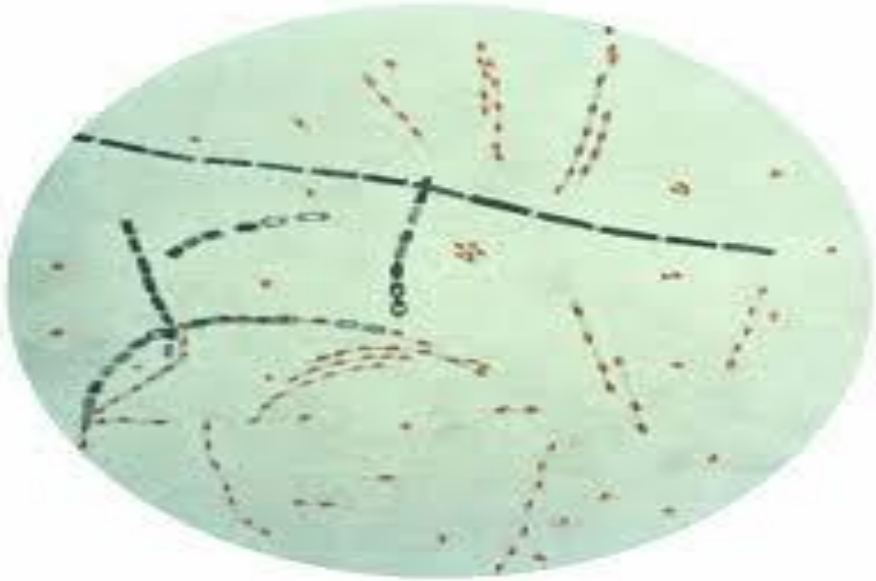
Domain: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Bacillaceae

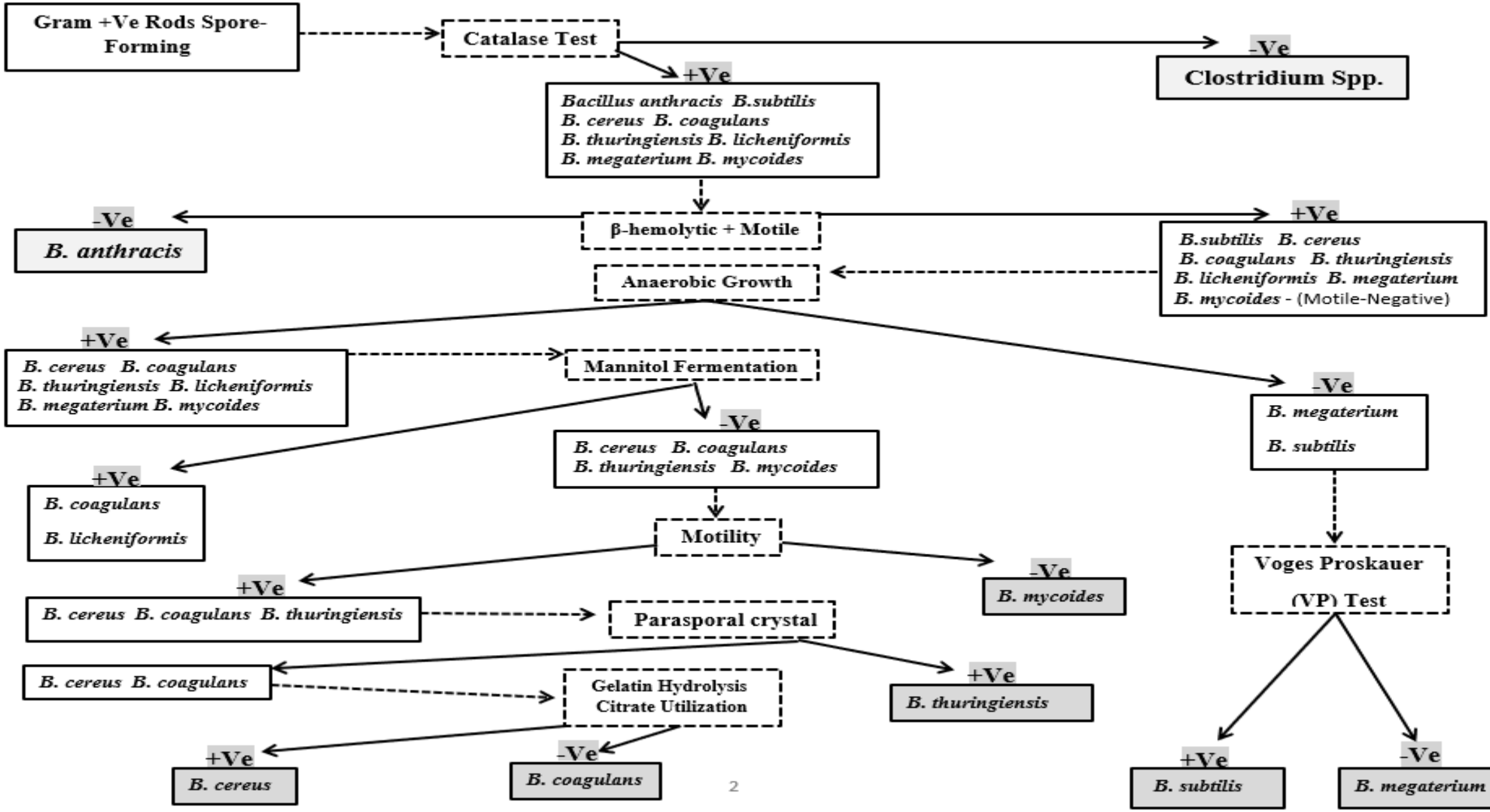


Genus: *Bacillus* . . . . . include at least 266 species.

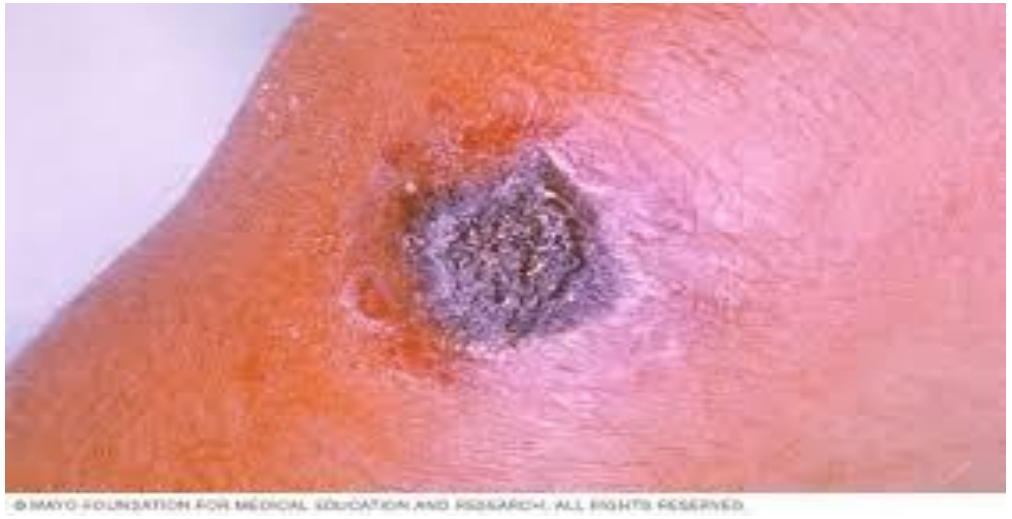
Species: *B. anthracis*

B. cereus

B. subtilis


Gram +Ve Rods Spore-Forming
Catalase Test
-Ve
Clostridium Spp.
+Ve
Bacillus anthracis B.subtilis
B. cereus B. coagulans
B. thuringiensis B. licheniformis
B. megaterium B. mycoides
-Ve
B. anthracis
β-hemolytic + Motile
+Ve
B.subtilis B. cereus
B. coagulans B. thuringiensis
B. licheniformis B. megaterium
B. mycoides - (Motile-Negative)
Anaerobic Growth
+Ve
B. cereus B. coagulans
B. thuringiensis B. licheniformis
B. megaterium B. mycoides
Mannitol Fermentation
-Ve
B. megaterium
B. subtilis
-Ve
B. cereus B. coagulans
B. thuringiensis B. mycoides
+Ve
B. coagulans
B. licheniformis
Motility
-Ve
B. mycoides
Voges Proskauer
(VP) Test
+Ve
B. cereus B. coagulans B. thuringiensis
Parasporal crystal
B. cereus B. coagulans
Gelatin Hydrolysis
Citrate Utilization
+Ve
B. thuringiensis
+Ve
B. cereus
-Ve
B. coagulans
+Ve
B. subtilis
-Ve
B. megaterium


gettyimages
Credit: Smith Collection/Gado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❖Endospore Staining

Materials per Student

1. 24- to 48-hour nutrient agar slant cultures of *Bacillus megaterium* and *Bacillus macerans.*
2. clean glass slides
3. hot plate or boiling water bath with staining rack or loop
4. 5% malachite green solution
5. safranin
6. bibulous paper
7. paper toweling

Procedure

1.Aseptically transfer one species of bacterium with an inoculating loop to each of the respective slides, air dry (or use a slide warmer), and heat-fix.

2. Place the slide to be stained on a hot plate or boiling water bath equipped with a staining loop or rack. Cover the smear with paper toweling that has been cut the same size as the microscope slide.

3. Soak the paper with the malachite green staining solution. Gently heat on the hot plate (just until the stain steams) for 5 to 6 minutes after the malachite green solution begins to steam. Replace the malachite green solution as it evaporates so that the paper remains saturated during heating. Do not allow the slide to become dry.

4. Remove the paper using forceps, allow the slide to cool, and rinse the slide with water for 30 seconds.

5. Counter stain with safranin for 60 to 90 seconds.

6. Rinse the slide with water for 30 seconds.

7. Blot dry with bibulous paper and examine under oil immersion. A coverslip is not necessary. The spores, both endospores and free spores, stain green; vegetative cells stain red.

https://www.youtube.com/watch?v=UchEPAJh4cs

Gelatin Hydrolysis

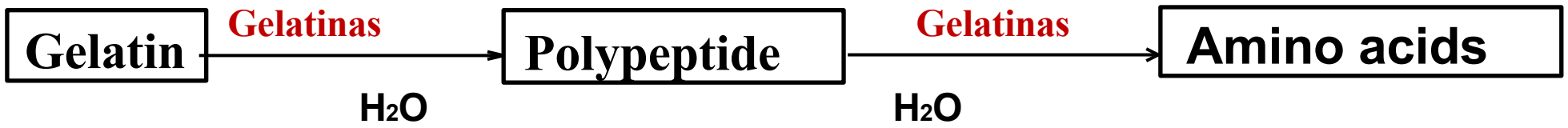
Gelatin is a protein derived from collagen, a component of vertebrate connective tissue. Gelatinases comprise a family of extracellular enzymes produced and secreted by some microorganisms to hydrolyze gelatin. Subsequently, the cell can take up individual amino acids and use them for metabolic purposes.

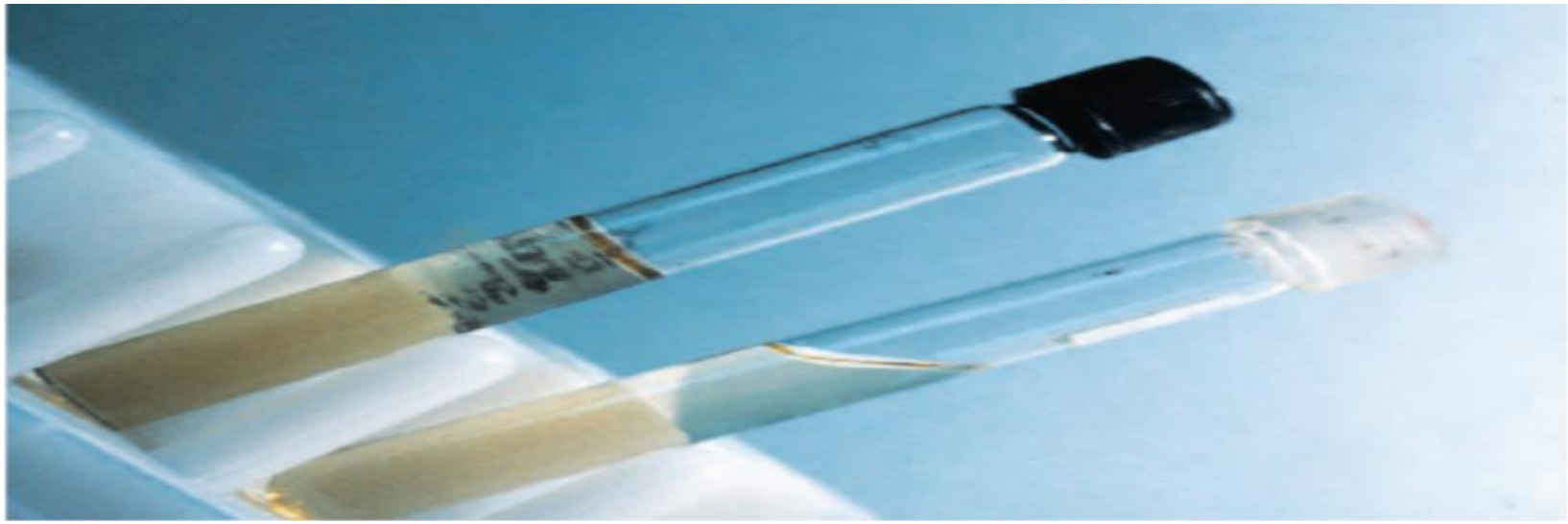
https://www.youtube.com/watch?v=TLLBUbGCpuw

The presence of gelatinases can be detected using **Nutrient Gelatin**, a simple test medium composed of gelatin, peptone, and beef extract. Nutrient Gelatin differs from most other solid media in that the solidifying agent (gelatin) is also the substrate for enzymatic activity. Consequently, when a tube of Nutrient Gelatin is stab-inoculated with a gelatinase positive organism, secreted gelatinase (or gelatinases) will liquefy the medium. Gelatinase-negative organisms do not secrete the enzyme and do not liquefy the medium.

Materials

1. 24- to 48-hour tryptic soy broth cultures of *Enterobacter aerogenes , Escherichia coli* and *Proteus vulgaris.*
2. 4 nutrient gelatin deep tubes.
3. 1-ml pipettes with pipettor.
4. refrigerator or ice-water bath.
5. test-tube rack.
6. incubator set at 35 °C.


Gelatin
Gelatinas
Polypeptide
Gelatinas
Amino acids
H2O
H2O



Procedure

1. Label three nutrient gelatin deeps with your name, date, and the bacterium to be inoculated. Label the fourth tube “control.”
2. Using aseptic technique , inoculate three of the deeps with the appropriate bacterium by stabbing the medium f of the way to the bottom of the tube.
3. Incubate the four tubes for 24 to 48 hours or longer at 35°C. The incubation time depends on the species of bacteria; some may require incubation for up to 2 weeks. If the latter is the case, observe on days 7 and 14.
4. Remove the nutrient gelatin deep tubes from the incubator and place them in the refrigerator at 4°C for 30 minutes or in an ice bath for 3 to 5 minutes.
5. When the bottom resolidifies, remove the tubes and gently slant them. Notice whether or not the surface of the medium is fluid or liquid. If the nutrient gelatin is liquid, this indicates that gelatin has been hydrolyzed by the bacterium. If no hydrolysis occurred, the medium will remain a gel. The uninoculated control should also be negative.

Second method without refrigerator

((A 7- day incubation period is usually sufficient to see liquefaction of the medium. However, gelatinase activity is very slow in some organisms. All tubes still negative after 7 days should be incubated an additional 7 days. A slight disadvantage of Nutrient Gelatin is that it melts at 28°C. Therefore, inoculated stabs are typically

incubated at 25°C along with an uninoculated control to verify that any liquefaction is not temperature-related.))