Laboratory

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Ahlulbait university

College of Pharmacy

Medical Microbiology **Class : 2nd**

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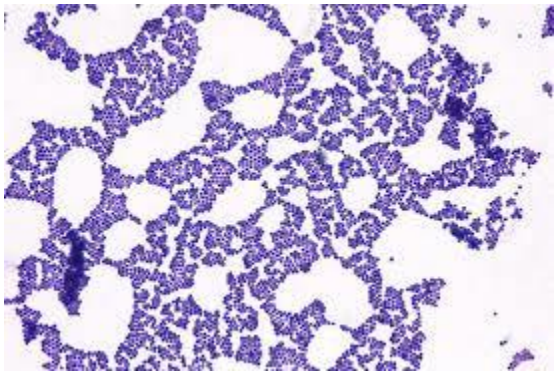
Staphylococcus



Staphylococci are Gram-positive cocci about 0.5 – 1.0 μm in diameter. They grow in clusters, pairs and occasionally in short chains. *Staphylococcus* is a genus often found as normal human microbiota of the skin and nasal cavity.

Mannitol salt agar or MSA:

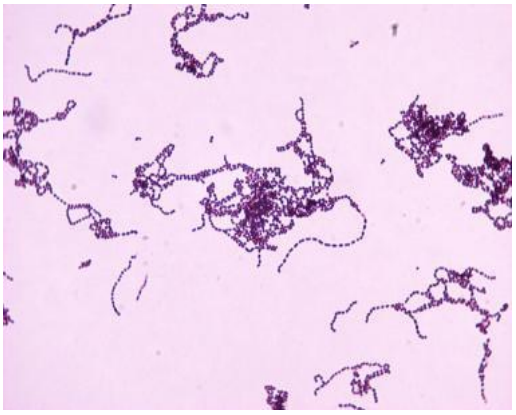
is a commonly used selective and differential growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time. It contains a high concentration (about 7.5%-10%) of salt (NaCl), making it selective for Gram-positive bacteria (***Staphylococcus*** and ***Micrococcaceae***) since this level of salt is inhibitory to most other bacteria

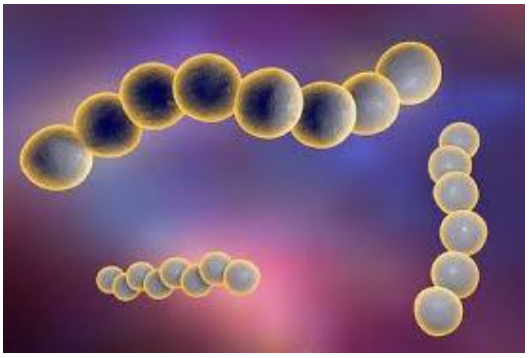
It is also a differential medium for mannitol-fermenting staphylococci, containing carbohydrate mannitol and the indicator phenol red, a pH indicator for detecting acid produced by mannitol-fermenting staphylococci.

Staphylococcus aureus produces yellow colonies with yellow zones, whereas other coagulase-negative staphylococci produce small pink or red colonies with no colour change to the medium.

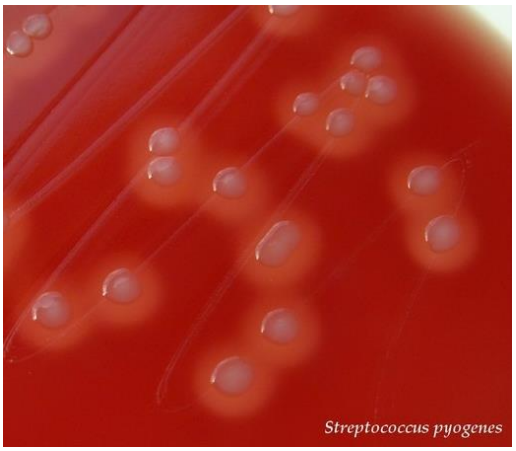
Streptococcus

Streptococci





Streptococci is a Gram-positive, nonmotile, nonsporeforming coccus that occurs in chains or in pairs of cells, catalase-negative, They are part of the **normal flora** of humans and animals. Some of them are human pathogens. The most important of them is ***Streptococcus pyogenes*** causing **pyogenic** Infections.



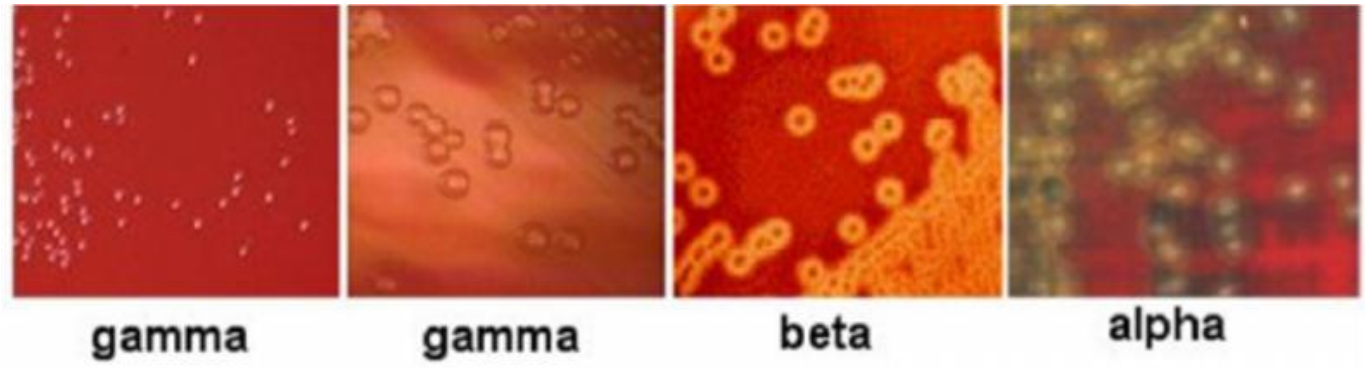
Culture Characteristics : It is an aerobes & facultative anaerobes growing best at 37°C (range 22 – 42). It is exacting in nutritive requirements, growth occurring in media containing fermentable carbohydrate & enriched with blood & serum.

When cultured on blood agar it gives colonies which are circular, low convex disc with area of clear hemolysis around it.

Hemolytic reactions

Blood agar : is a common medium used to culture bacteria because,1- it is a great enrichment medium for **fastidious** bacteria, and 2) hemolysis of blood cells can be very useful as an identification test. Blood agar is made with 5% sheep blood.

Hemolysis is the breakdown of red blood cells: hemolysins are enzymes produced by some bacteria and are released into the medium around the bacterial colony. It can be a complete breakdown of
the cells, with the release of hemoglobin and a clearing of the red from the surrounding medium
around the colony. Or the hemolysis can be a partial breakdown, resulting in a greenish or green-yellow zone around the colony.



Hemolytic reactions

- alpha (α) hemolysis – green zone around colony, caused by leaking hemoglobin converted to biliverdin, called a partial hemolysis

- beta (β) hemolysis – complete clearing around colony caused by breakdown of RBCs by streptolysin enzymes.

- gamma (γ) hemolysis - no hemolysins, no zone.

Staphylococcus species are either beta hemolytic or gamma (not hemolytic). *Staph aureus* produces alpha toxin which typically causes wide zones of beta (complete) hemolysis.

Catalase Test

Short review:

The bacteria produce hydrogen peroxide (H_2O_2) during their aerobic respiration, and if it accumulates inside the bacterial cells, it s too toxic, so usually most bacteria (aerobic & facultative anaerobic) will utilize this enzyme to degrade H_2O_2**.**

Principle:

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculums is introduced into hydrogen peroxide (3% solution), and a rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production.

Method:

Use a loop or sterile wooden stick to transfer a small amount of colony growth to the surface of a clean, dry glass slide. Place a drop of **3% hydrogen peroxide (**H_2O_2**)** onto the inoculums. Observe for the evolution of oxygen bubbles.

Expected Results:

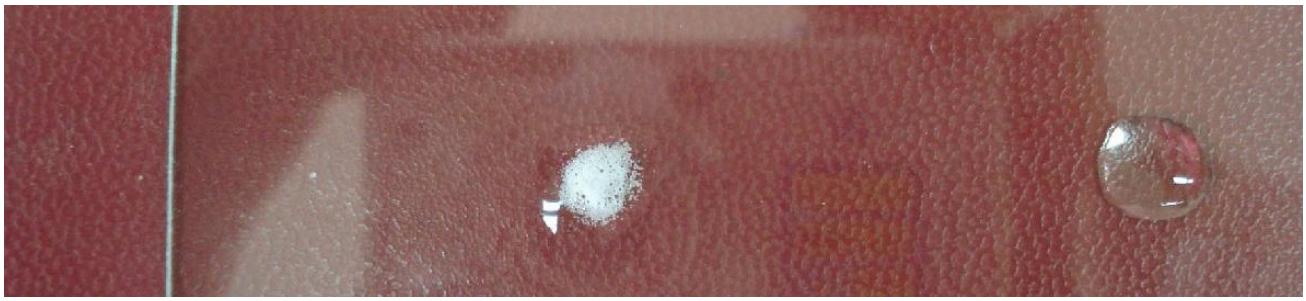
Catalase-positive organisms (e.g., ***staphyloccoci, Listeria monocytogenes***, and ***corynebacterium*** spp.) produce copious bubbles; catalase-negative organisms (e.g., *streptococci* and enterococci) yield no or few bubbles.

Note: some bacteria produce peroxides that slowly catalyzes the breakdown of (H_2O_2) and the test may appear weakly positive (a few bubbles slowly elaborated). This reaction is not a truly positive test and is considered negative.

Notes to be observed:

1- Don t use media containing blood, because the red blood cells contain Catalase and it will give us a false positive test.

2-Always use a fresh H_2O_2 because it s unstable and check it via a control known strain of a Catalase positive one.



Revealed a positive catalase test regarding *Staphylococci* spp. Isolated from impetigo case (left) and a negative result regarding *Streptococcus* spp.

Coagulase Test

Short review:

This test is used to differentiate ***Staphylococcus aureus*** (positive) from Coagulase negative staphylococci (negative). ***S. aureus*** produced two forms of Coagulase: bound and free. **Bound Coagulase** or "**clumping factor**" is bound to the bacterial cell wall and reacts directly with **fibrinogen**. This results in an alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma.

Principle:

Clumping factor directly converts **fibrinogen** to fibrin causing agglutination.

There are two methods for Coagulase test to be carried out:

1- Slide coagulase test is done to detect bound coagulase or clumping factor.

2- Tube coagulase test is done to detect free coagulase.

Procedure:

1. Divide the slide into two sections with grease pencil. One should be labeled as “test” and the other as “control.
2. a small drop of distilled water on each area.
3. Emulsify one or two colonies of Staphylococcus on blood agar plate on each drop to make a smooth suspension.
4. The test suspension is treated with a drop of citrated plasma and mixed well with a needle.
5. Do not put anything in the other drop that serves as control. The control suspension serves to rule out false positivity due to auto agglutination.
6. Clumping of cocci within 5-10 seconds is taken as positive.
7. Some strains of S.aureus may not produce bound coagulase, and such strains must be identified by tube coagulase test.