Laboratory

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Ahlulbait university

College of Pharmacy

Medical Microbiology **Class : 2 nd**

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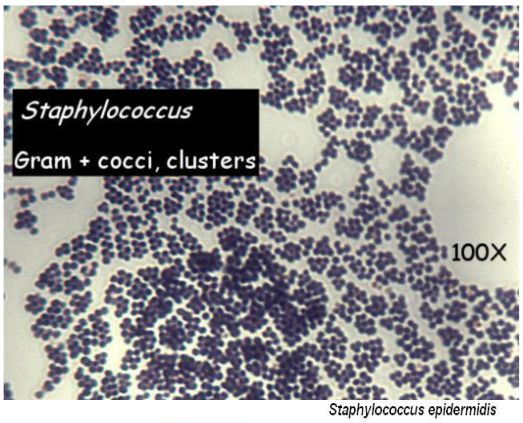
Staphylococcus



Staphylococci are Gram-positive cocci about 0.5 – 1.0 μm in diameter. They grow in clusters, pairs and occasionally in short chains.. The configuration of the cocci helps to distinguish micrococci and staphylococci from streptococci, which usually grow in chains.

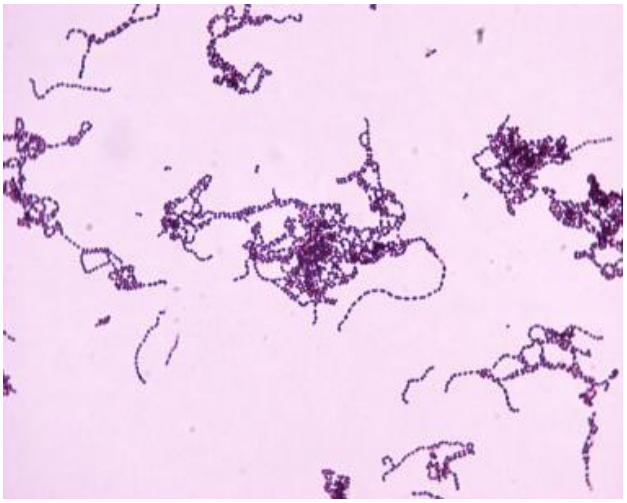
Mannitol salt agar or MSA: is a commonly used selective and differential growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period 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. [3] ***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.

S. aureus is catalase-positive (meaning it can produce the enzyme catalase). Catalase-activity tests are sometimes used to distinguish staphylococci from enterococci and streptococci. Previously, *S. aureus* was differentiated from other staphylococci by the coagulase test.
Staphylococcus is a genus often found as normal human microbiota of the skin and nasal cavity.

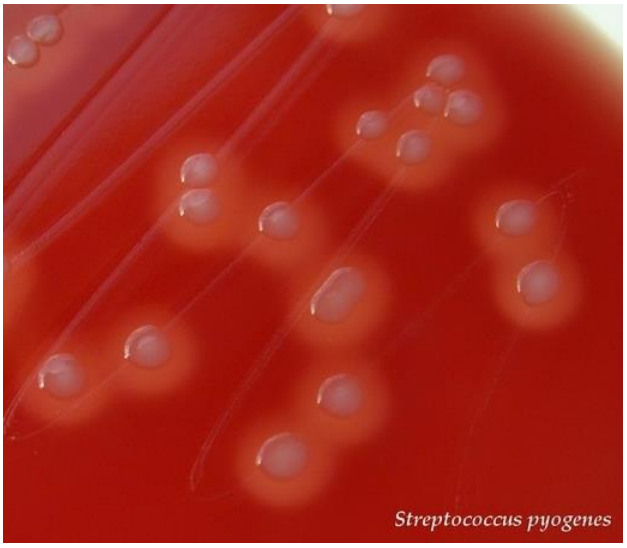
STREPTOCOCCUS

Streptococci



Streptococci are Gram-positive cocci arranged in chains or pairs. 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.

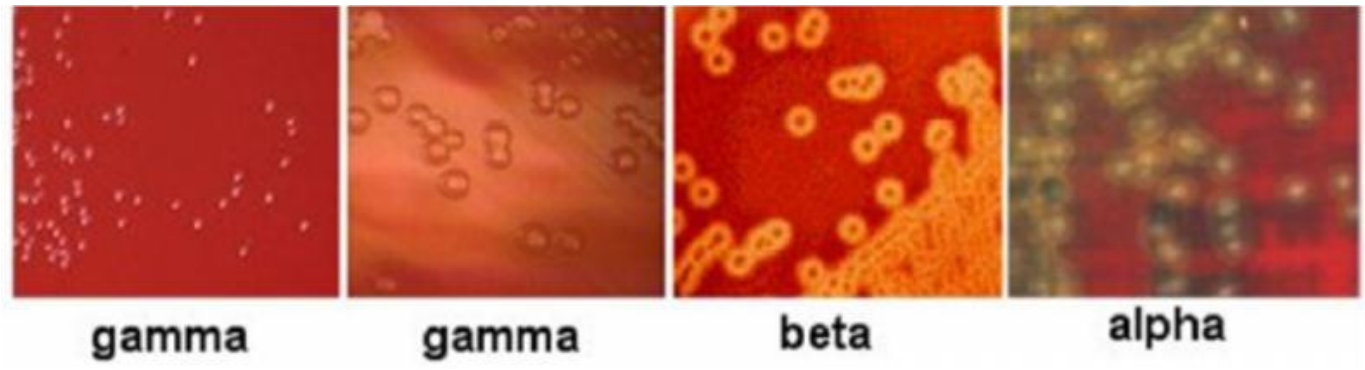
Culture Characteristics : It is an aerobes & facultative anaerobes growing best at 37°C (range 22 – 42). It is exacting in nutritive requirements, growth occurring in media containing fermentable carbohydrate & enriched with blood & serum.
When cultured on blood agar it gives colonies which are circular, low convex disc with area of clear hemolysis around it.



Hemolytic reactions

Blood agar (BAP) 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:

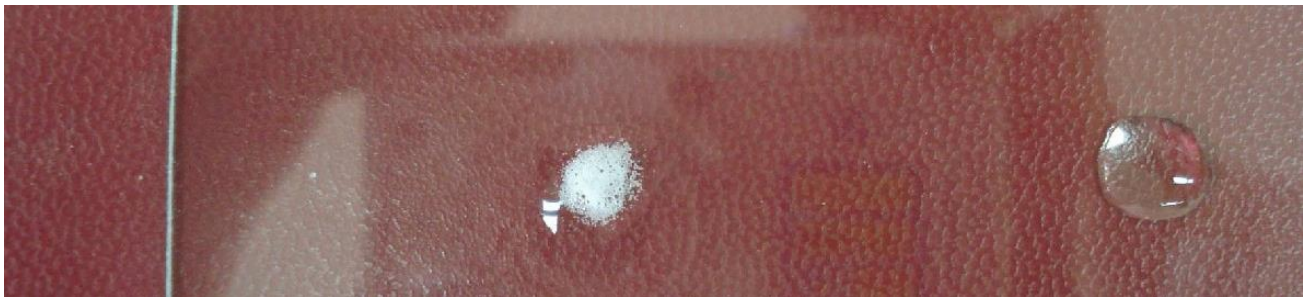
Catalase-positive organisms (e.g., ***staphyloccoci, Listeria monocytogenes***, and ***corynebacterium*** spp.) produce copious bubbles; catalase-negative organisms (e.g., *streptococci* and enterococci) yield no or few bubbles.

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:

The presence of bound Coagulase correlates well with free Coagulase, an extracellular protein enzyme that causes the formation of a clot when ***S. aureus*** colonies are incubated with plasma. The clotting mechanism involves activation of a plasma Coagulase- reacting factor (CRF), which is modified or derived thrombin molecules, to form a Coagulase-CRF complex. This complex in turn reacts with fibrinogen to produce the fibrin clot.

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

1- Slide method.(slide Coagulase test for free clumping factor only)
2- Tube method.(tube Coagulase test for free and bound clumping factor)

The Slide coagulase test

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.