

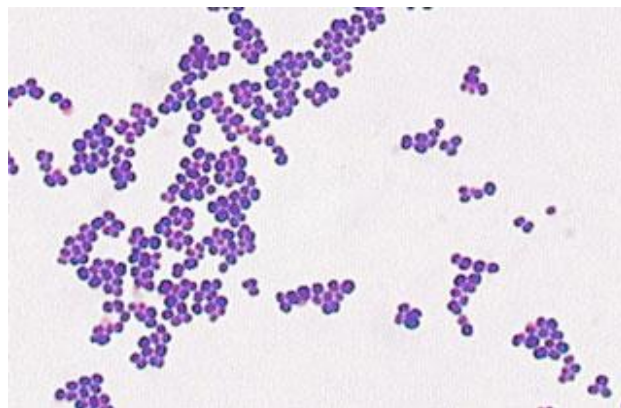
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## *Staphylococci*

*Staphylococci* are members of the Micrococcaceae family. They are classically considered extracellular, pyogenic pathogens because of their ability to induce abscess formation. The bacteria are Gram positive, non-motile and tend to grow in clusters (grape like cluster). They are extremely hardy and can survive for prolonged periods of time on environmental surfaces. This family includes *Staphylococcus aureus*, *epidermidis* and *saprophyticus*.

### **Gram Stain**

Gram positive cocci (purple), and found as either single cells, in pairs, or more frequently, in clusters that resemble a bunch of grapes., Approximately 1µm in diameter. The genus name *Staphylococcus* is derived from Greek terms (staphyle and kokkos) that mean "a bunch of grapes,"



### ***Staphylococci* on Blood Agar**

Blood agar is both differential and enriched medium. The blood that is incorporated into this medium is an enrichment ingredient for the cultivation of fastidious organisms. On blood agar, *S. aureus* usually displays a light to golden yellow pigment, whereas *S. epidermidis* has a

white pigment and *S. saprophyticus* either a bright yellow or white pigment. However, pigmentation is not always a reliable characteristic. On blood agar, *S. aureus* is usually beta hemolytic (complete lysis of blood), *S. epidermidis* and *S. saprophyticus* are almost always non hemolytic.



*S. aureus* (left) and *S. epidermidis* (right)

## Mannitol Salt Agar

Mannitol salt agar (MSA) is both a selective and differential media used for the isolation of Staphylococci from mixed cultures.

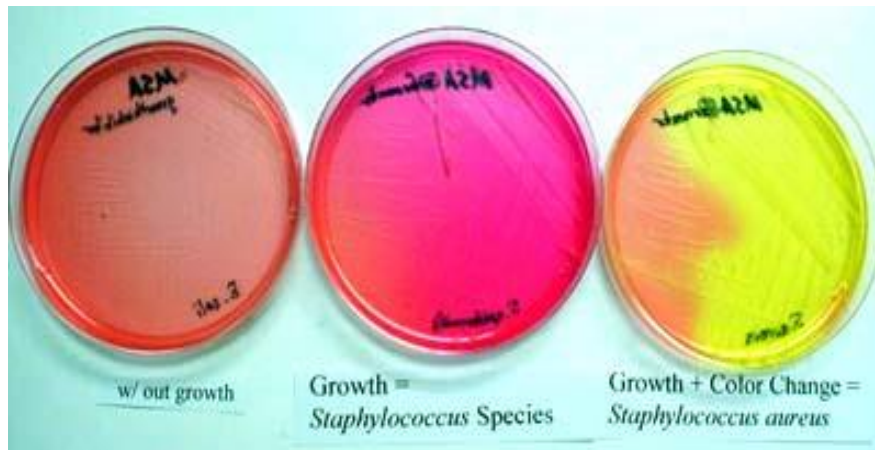
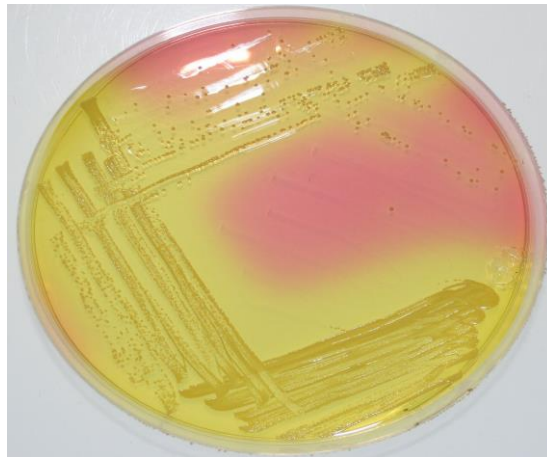
### MSA components

**7.5% NaCl** – selects for species of *Staphylococcus*. This concentration of salt is too high for most other bacteria to withstand and, therefore, inhibits their growth.

**Mannitol** – sugar alcohol of the carbohydrate mannose. Mannitol fermentation produces acid end products which turn the medium yellow. Yellow indicates mannitol positive and no color change indicates mannitol negative.

**Phenol red pH indicator** – yellow in acid pH (the same indicator that is used in phenol red carbohydrate fermentation broths).

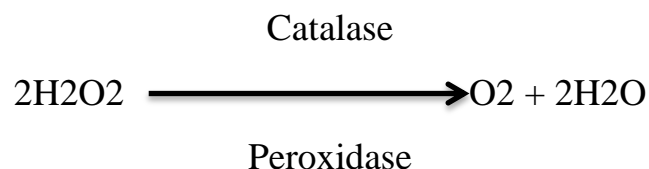
On MSA, only pathogenic *Staphylococcus aureus* produces small colonies surrounded by yellow zones. The reason for this color change is that *S. aureus* have the ability to ferment the mannitol, producing an acid, which changes the indicator color from red to yellow. The growth of other types of bacteria is usually inhibited. This growth differentiates *S. aureus* from *S. epidermidis*, which forms colonies with red zones.



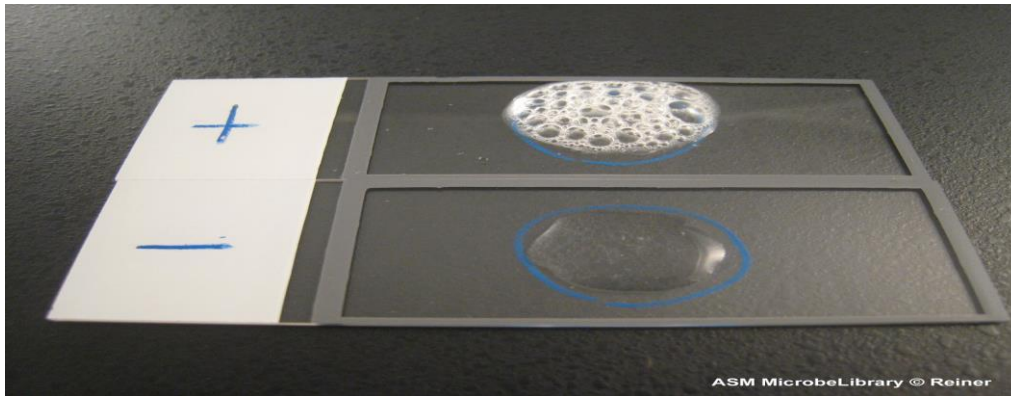
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**Biochemical reactions:****1- Catalase test:**

Some bacteria contain flavoproteins that reduce oxygen (O<sub>2</sub>), resulting in the production of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Accumulation of these substances will result in death of the organism as they are powerful oxidizing agents and destroy cellular constituents very rapidly unless they can be enzymatically degraded. Many bacteria possess enzymes that afford protection against toxic H<sub>2</sub>O<sub>2</sub> products, either *catalase* or *peroxidase*, which catalyze the destruction of hydrogen peroxide as follow

**Procedure of catalase test (Slide Test)**

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
2. Place a drop of 3% H<sub>2</sub>O<sub>2</sub> on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.



## 2- Coagulase test

Coagulases are enzymes that clot blood plasma by a mechanism that is similar to normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease causing bacteria of humans that produce coagulase enzyme are *Staphylococcus aureus*. Thus this enzyme is a good indicator of the pathogenic potential of *S. aureus*.

Most strains of *S. aureus* produce one or two types of coagulase; **free coagulase** and **bound coagulase**. Bound coagulase is localized on the surface of the cell wall and reacts with  $\alpha$ - and  $\beta$ -chains of the plasma fibrinogens to form a coagulate. Free coagulase is an enzyme that is secreted extracellularly. Free coagulase can be detected in tube coagulase test and bound coagulase can be detected in slide coagulase test. Free coagulase is always heat labile while bound coagulase is heat stable.

### Detection of bound coagulase - Slide Test

This method measures bound coagulase. The bound coagulase is also known as clumping factor.

1. Divide the slide into two sections with grease pencil. One should be labeled as „test” and the other as „control“.

2. Place 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.

