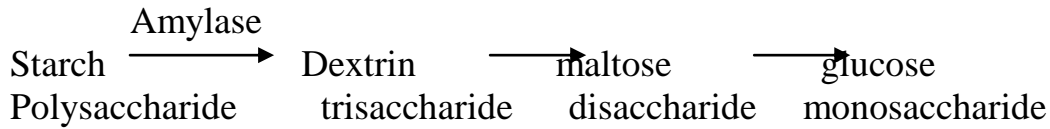


6- Starch hydrolysis test.

❖ mechanism of action or mode of action:-



Used to this purpose starch agar and pH indicator (Gram's Iodine)

Starch medium:- (للاطلاع)

Starch agar 5gm
Distilled water 100ml

Autoclaving and pour the medium in plates then culture the bacteria by stabbing or spreading by small circular. Incubate at 37°C° for 24 hour.

Result in this test:- Add drops from iodine grams to culture media **Brown colonies** with present clear zone from hydrolysis around colonies (+) e.g. *Bacillus subtilis*
Blue colonies without present clear zone from hydrolysis around colonies (-)

7- Casein hydrolysis test :-

❖ mechanism of action or mode of action:-

Casein is the principal protein of milk. It exist as a colloidal suspension that gives milk it's opaque whitness. Many bacteria are equipped with enzymes (caseinase) that hydrolyze this protein (caseine) into more soluble and transparent derivatives. This called process peptonization. Used to this purpose skim milk agar.

Caseine medium:- (للاطلاع)

Skim milk agar 3gm
Distilled water 100ml

Autoclaving and pour the medium in plates then culture the bacteria by spreading method by small circular. Incubate at 37°C° for 24 hour

Result in this test :-

Clear zone around colonies (+) e.g *Bacillus subtilis*, *Pseudomonas*
Non clear zone around colonies (-)

8- Gelatin hydrolysis test .

❖ mechanism of action or mode of action:-

The test used to distinguish some types of bacteria which have the ability to secrete some enzymes (gelatinase) which hydrolyse gelatin. Considered major components of connective tissue and tendons.

Gelatin medium:- (للاطلاع)

Nutrient agar 1.3 gm
Gelatin 12%
Distilled water 100ml

Autoclaving and distribution in the sterile test tubes then inoculate tube of Nutrient gelatin with bacteria and incubate at 37 C° with sterile tube of Nutrient gelatin that will serve as control for 2-7 days.

Result in this test:-To examine for hydrolysis, chill the tubes in ice water in the refrigerator for 3 minutes. Hydrolysed gelatin will remain fluid (+) .
Unhydrolysed gelatin has taken place will solidify (-).

9- Litmus milk .

❖ mechanism of action or mode of action:-

Milk indicates both saccharolytic and proteolytic properties of bacteria by detecting whether they ferment lactose or digest casein. Used to this purpose (skim milk) and pH indicator (litmus stain)

Litmus milk medium (للاطلاع)

Litmus milk 10 gm
Litmus stain 0.075 ml
Distilled water 100ml

Autoclaving 10 minutes and distribution in sterile test tubes then inoculate tubes with bacterial and incubate at 37 C° for 24 hours.

The result in this test :-

1-Acid production :-lactose fermenters in litmus milk form acid which lead to decrease in pH that change to pink color. Large amounts of acid will precipitate the casein as a clot. e.g *Corynebacterium*.

2-Reduction:- white top Layer to the Litmus milk (+) .resulte in ability to reduced bacteria to the removal O₂ from milk e.g *pseudomonase*.

3-Rennet curd and peptonization :- yellow liquid like straw(+). which produce from proteolytic bacteria may decompose milk proteins to a transparent solution of soluble products. e.g. *Streptococcus fecalis*.

4-Alkaline:-blue color (+). Result in ability of some types bacteria to removal CooH and NH₄ from casein amino acid e.g *Staphylococcus aureus*.

5-Gas and acid production:- (+) gas formed during coagulation the clot will be distributed by it (stormy clot) e.g. clostridium

10- Coagulase test .

❖ mechanism of action or mode of action:-

This test used to distinguished between pathogenic strains and non-pathogenic strains *staphylococcus*. Most strain of *Staphylococcus aureus* produce free coagulase which is detect by tube coagulation and bound coagulase or clumping factor which is detected by slide coagulase test. Used to this purpose human and rabbit blood plasma.

There are two procedure method to detect activity coagulase.

1-Slide coagulase test :- a loopful of the *staphylococcal* Culture is emulsified in drops of water on slide then another loopful of rabbit plasma is added and mixed well with the bacterial suspension. The slide is gently rocked and a positive reaction is indicated by clumpng within one or two minutes.

2-Tube coagulase test :- 0.1 ml of an over night colonies from solid media and added to the 1ml of diluted plasma (1:10). The tube is rotated gently to mix the contents and then incubated at 37 C°. A positive test with clotting of plasma and occur in 2-4 hours. However many weak coagulase -positive strains will coagulate the plasma only after over night incubation.