STUDY THE INFLUENCE OF ENVIRONMENTAL FACTORS ON MICROBIAL GROWTH

- Biological factors
- physical factors
- chemical factors

Biological factors(Microbial enzymes)

Microbial enzymes it is a substance secreted by some microorganisms that has the ability to accelerate metabolic reactions as it decomposes a lot of complex carbohydrate, protein and fatty substances into small molecules that can be absorbed.

Protease enzyme

An experiment: testing the ability of bacteria to analyze protein to produce the enzyme Protease.

The protease enzyme is considered one of the external internal enzymes that degrade proteins. The internal enzymes are the enzymes that are not secreted into the medium except after cell decomposition, while the external enzymes are secreted into the medium naturally without cell lysis.

It was observed that the protease enzymes produced from micro-organisms play a large and important role in the resistance of these organisms to the .abnormal conditions and in the diseases that they cause.

Protease enzymes break down proteins of various kinds, such as casein, hemoglobin, etc.

The aim of the experiment: To distinguish between protease-producing and non-producing bacteria

Materials:

1-Isolation of the protease-producing Bacillus bacteria

2-Skim milk, sterilized

3-Sterile Petri dishes

4-Nutrient agar

The method of work:

1- 100ml of nutrient agar medium is prepared and sterilized with the autoclave, then add 3% of the sterilized skimmed milk to the autoclave separately, mix the contents well and pour into sterile dishes.

2-The plate is inoculated with a modern culture of *Bacillus* bacteria and incubated at 37 m for 24 hours

Results:

It reveals the degradation of the protease enzyme by forming transparent regions around the bacterial growth, evidence that the bacteria produced the enzyme to the outer medium and analyzed the milk proteins, but if transparent areas did not appear around the developing colonies, this is an indication of the inability of the bacteria to produce the enzyme and thus the failure of the milk to dissolve in the culture medium.

Catalase enzyme

The enzyme, catalase, is produced by bacteria that respire using oxygen, and protects them from the toxic by-products of oxygen metabolism. The catalase test is used to differentiate *staphylococci* (catalase-positive) from *streptococci* (catalase-negative). The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water.

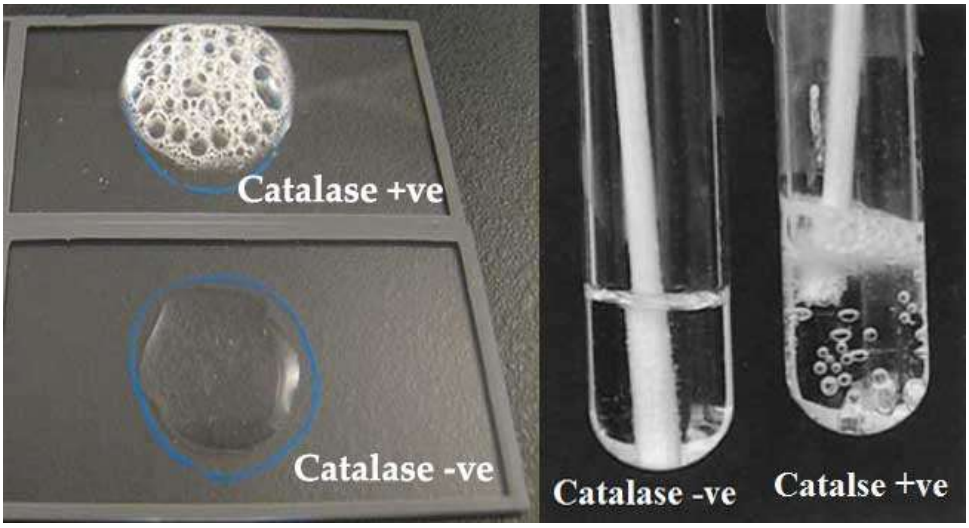
Procedure of Catalase Test

Tube Method

1. Pour 1-2 ml of hydrogen peroxide solution into a test tube.
2. Using a sterile wooden stick or a glass rod, take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution.
3. Observe for immediate bubbling.

Slide Method

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.
3. Observe for the evolution of oxygen bubbles.



Precautions of Catalase Test

- The test organisms should not be taken from blood agar culture. Red Blood cells contain catalase and their presence will give a false positive test.
- Culture should be 18 to 24 hours old.
- Hydrogen peroxide must be fresh as it is very unstable.
- Iron wire loop should not be used.
- Some bacteria produce a peroxidase that catalyzes a breakdown of hydrogen peroxide causing the reaction to be weakly positive; (a few bubbles elaborated slowly). This should not be confused with a truly positive reaction.
- Do not add organism to reagent, particularly if iron-containing inoculating loops are used. Iron containing loops will cause false positive test results if exposed to hydrogen peroxide.