MICROBIAL ENZYMATIC ACTIVITY

“ 240 MIC ”

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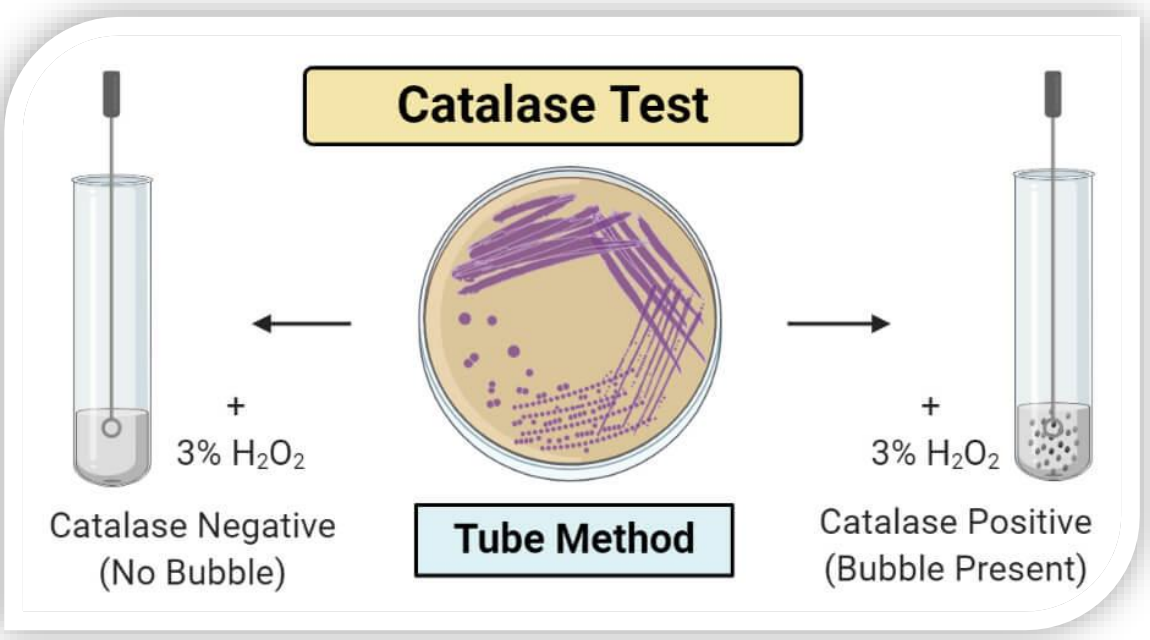
Bacteria accomplish their various biochemical activities (growth and multiplication) using raw materials (nutrients) obtained from the environment. The biochemical transformations that occur both inside and outside of bacteria are governed by biological catalysts called **enzymes.**



Kinds of bacterial enzymatic reactions

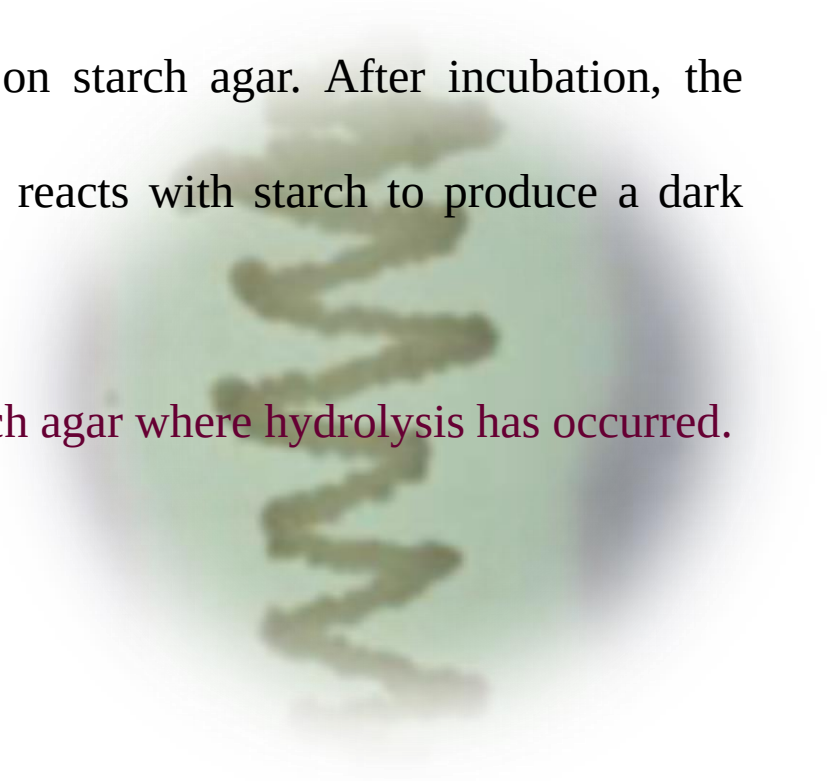
1. Hydrolysis of **starch** into maltose and glucose (ex: **Amylase**).
2. The breakdown of toxic wastes such as **hydrogen peroxide** or **urea** (ex: **Catalase**).
3. Hydrolysis of **protein** (casein) to amino acids (ex: **Proteases**).
4. The reduction of **nitrate** or **oxygen** (ex: **Nitrate reductase**).
5. The degradation of specific **amino acids** (ex: **Treptophanase**).
6. The utilization of **non-carbohydrate carbon** sources for growth (ex: **urease**).

AMYLASE PRODUCTION TEST

- ✓ **Amylase is an exoenzyme that hydrolyses starch**.

Starch is a polysaccharide--a long chain of glucose molecules linked by glycosidic bonds.

- ✓ Amylase breaks the glycosidic bonds (α- 1,6-glucosidase), producing small oligosaccharides and free glucose.
- ✓ Amylase production is tested by growing organisms on starch agar. After incubation, the starch agar is flooded with Gram's iodine. The iodine reacts with starch to produce a dark purple or brown color.
- ✓ If amylase is present, clear zones will appear in the starch agar where hydrolysis has occurred.



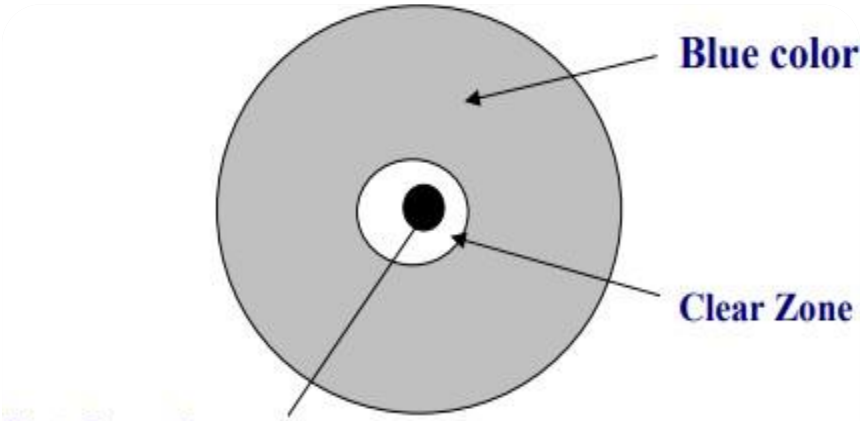
PROCEDURE

1. Streak each organism across a small portion of the starch agar surface.

2. Incubate at 37 ^{o} C for 48 hours.

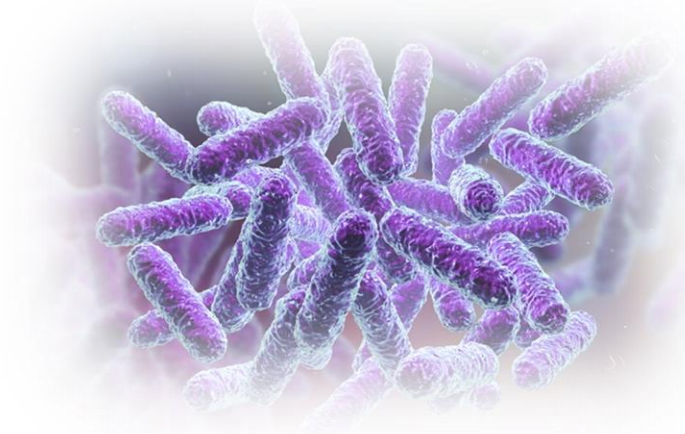
3. Cover the surface with **iodine**.
Rotate to distribute the iodine into a thin layer. Do not flood the plate. Record your results.

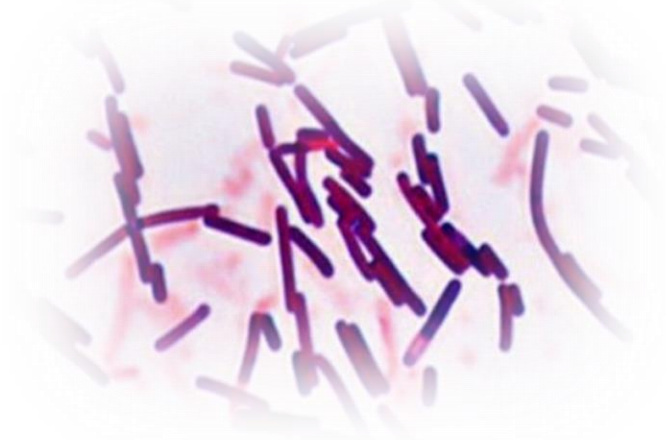
4. **Iodine** will turn **blue** when it reacts with starch.
A clear zone will be seen where starch has been digested.

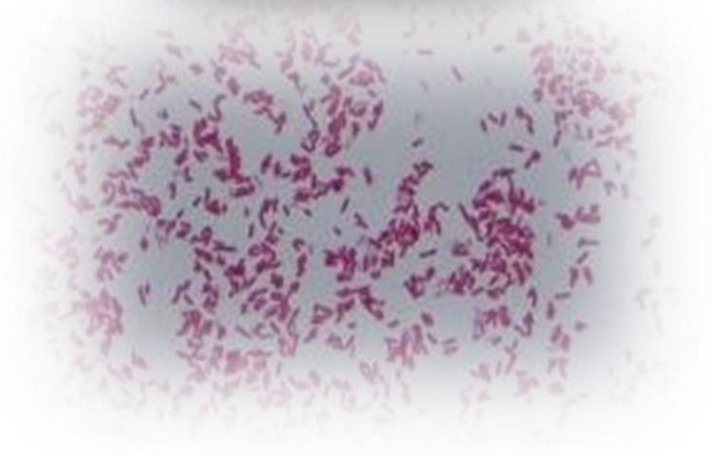


Result of Amylase Production

Result of Amylase Production	
Positive	**Negative**
Bacillus subtilits	***E.coli***

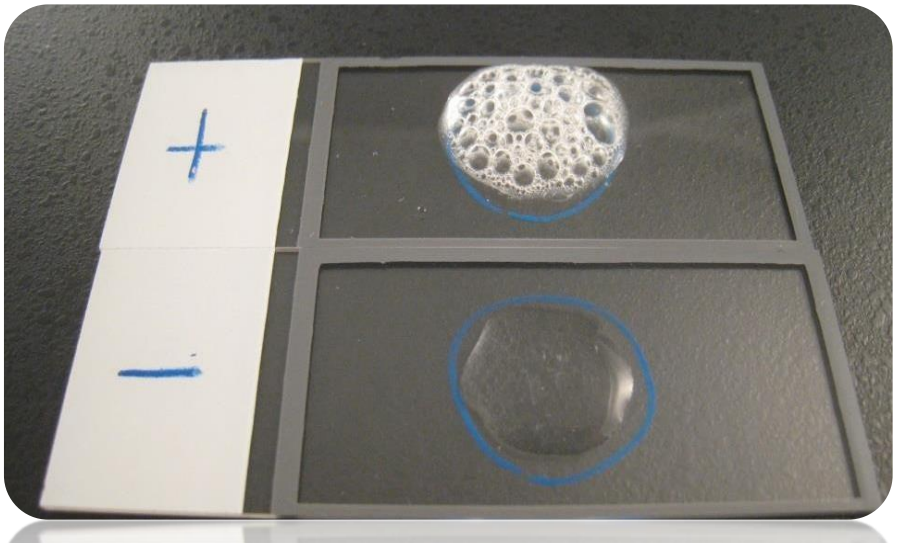






CATALASE ACTIVITY

- ✓ Catalase production and activity can be detected by adding the substrate H_2O_2 to an appropriately incubated (18- to 24-hour) tryptic soy agar slant culture.
- ✓ If catalase was produced by the bacteria, the above chemical reaction will liberate free O_2 gas. Bubbles of O_2 represent a positive catalase test; the absence of bubble formation is a negative catalase test.
- ✓ Catalase activity is very useful in differentiating between groups of bacteria. For example, the morphologically similar *Enterococcus* (catalase negative) and *Staphylococcus* (catalase positive) can be differentiated using the catalase test.

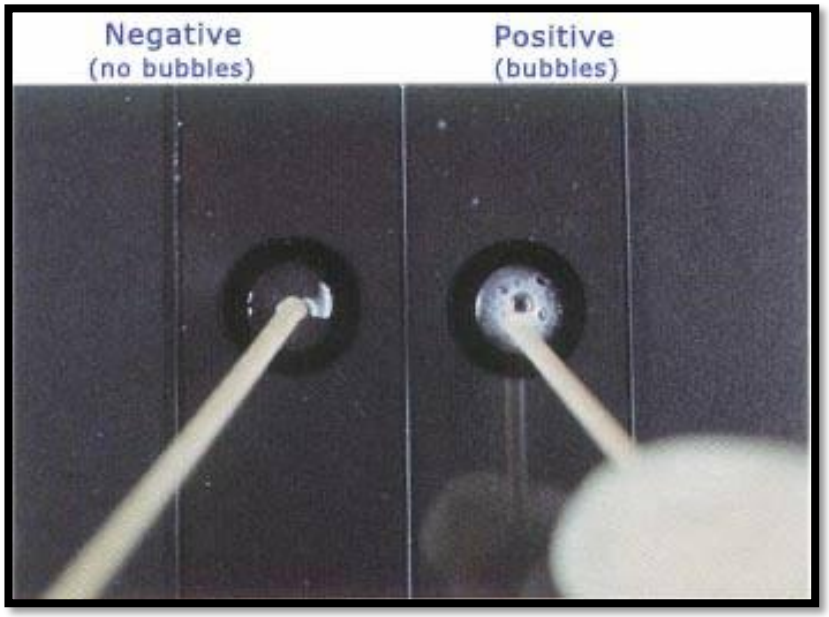


THEORY

The catalase enzyme serves to neutralize the bactericidal effects of hydrogen peroxide.

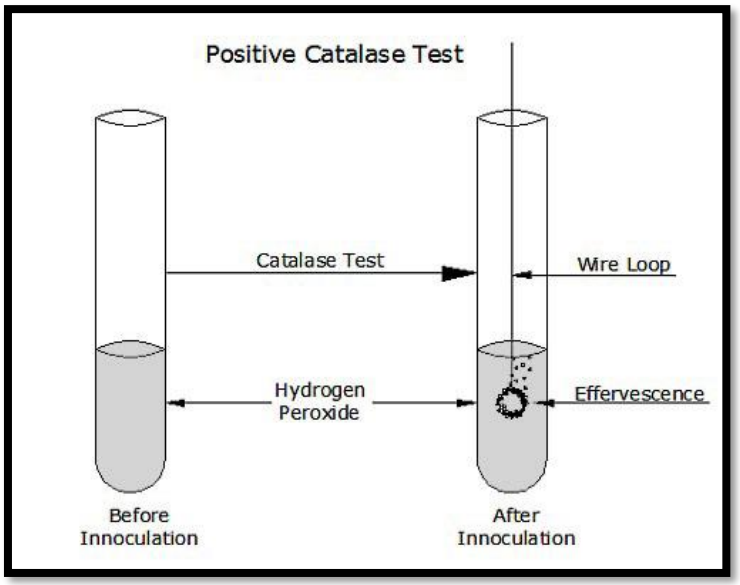
Catalase expedites the breakdown of hydrogen peroxide (H_2O_2- (toxic) into water (H_2O) and oxygen (O_2)

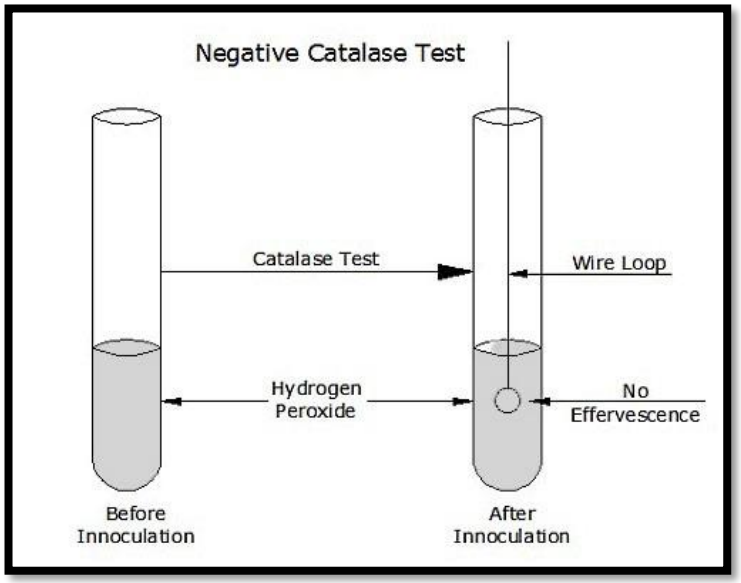
$2H_2O_2$ + Catalase → $2H_2O$ + O_2 (gas bubbles)



CATALASE TEST

- ✓ The catalase test is used to detect the presence of the **enzyme catalase** in bacteria.
- ✓ Catalase serves to neutralize the bactericidal effects of **hydrogen peroxide.**
- ✓ This enzymatic test is essential in the scheme of identification for gram-positive organisms and certain gram-negative organisms.
- ✓ It is a primary test used in the differentiation of staphylococci and streptococci.





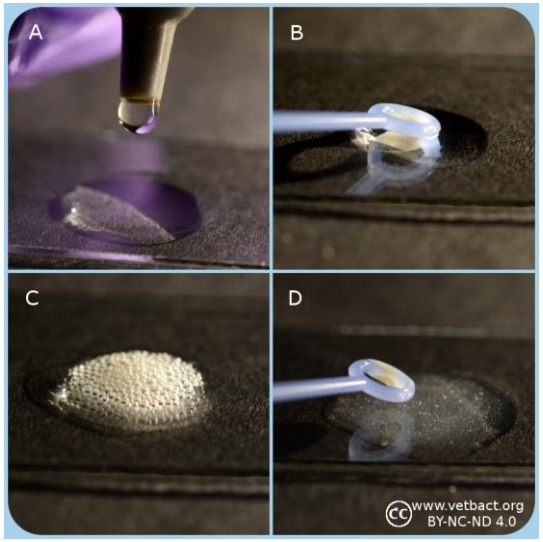
PROCEDURE

1. Label each of the tryptic soy agar slants with the name of the bacterium to be inoculated, your name, and date.

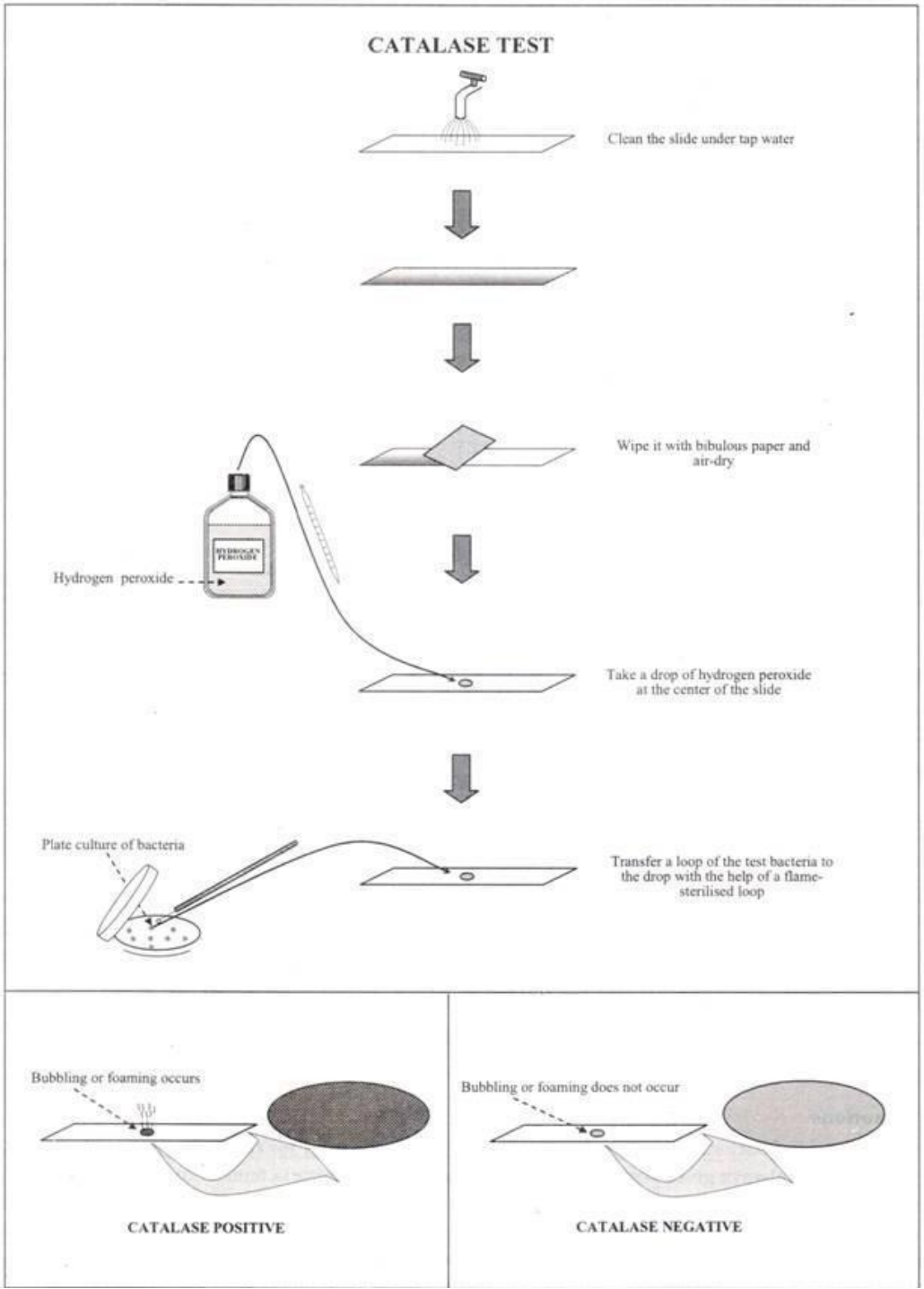
2. Using aseptic technique, heavily inoculate each experimental bacterium into its appropriately labeled tube by means of a streak inoculation.

3. Incubate the slants at 35°C for 18 to 24 hours.

4. Remove growth from a slant using a wooden applicator stick or Nichrome wire loop and place the growth on a glass slide. The cells are then mixed in a drop of 3% H_2O_2 or a drop of Difco's Spot Test catalase reagent.



Immediate bubbling indicates a positive catalase test.



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