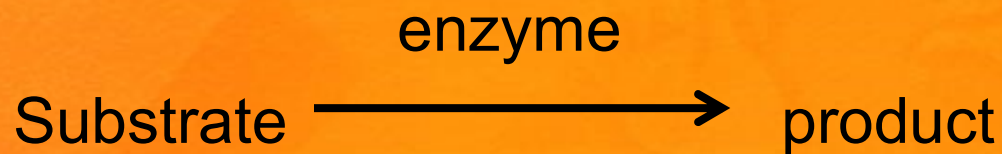


Introduction to Biochemical tests



- In the microbiology lab, biochemical test relies on enzymes which is glycoprotein or protein that act as catalyst by lowering the activation energy of certain biological reaction.



- We can use our knowledge in bacterial enzymes to identify the bacteria and distinguish between bacterial species.

Types of enzymes

1. According to site of the reaction

- a. Endoenzymes : where substrate and enzyme react inside the cell. (ex: oxidase, catalase, urease).
- b. Exoenzymes: where substrate and enzyme react outside the cell. (ex: free coagulase, gelatinase, amylase).

2. According to enzyme production

- a. inducible : produced only when needed or induced.
- b. constitutive : produced continuously

Notes

1. Every genus of bacteria has its unique set of enzymes, so we can identify it.
2. Endoenzymes may act outside the cell in case of the presence of high concentration of its substrate.
3. The same enzyme could be inducible and constitutive in different genera

Kinds of bacterial enzymatic reactions

1. The breakdown of toxic wastes such as hydrogen peroxide or urea (ex: catalase)
2. The reduction of nitrate or oxygen (ex: nitrate reductase).
3. The degradation of specific amino acids (ex: treptophanase).
4. The utilization of noncarbohydrate carbon sources for growth (ex: urease).

Catalase test



Background

- Enzyme name \ catalase
- Substrate name \ hydrogen peroxide
- Enzyme action \ breakdown the toxic H_2O_2 producing oxygen gas and water



- Hydrogen peroxide produce due to the aerobic respiration of the cells and have to be breakdown to prevent it's toxic action on DNA and cell membrane

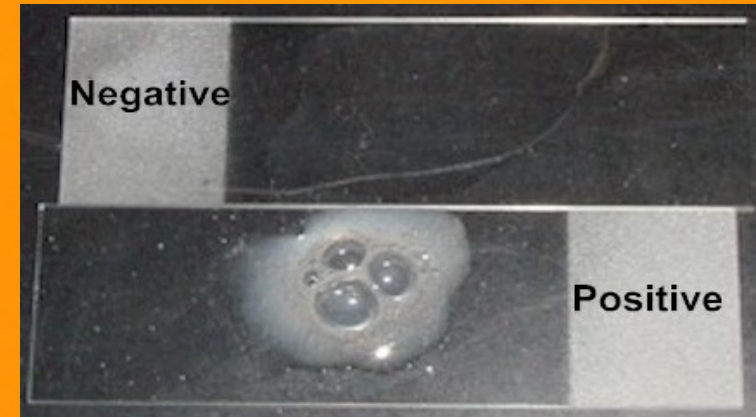
Background

- When hydrogen peroxide is added to a colony of catalase-producing bacteria, it is broken down and the oxygen that is produced can be seen as bubbles .
 - By catalase test we can distinguish between:
 - G (+ve) cocci : staphylococcus is catalase positive where streptococcus is catalase negative
 - G (+ve) bacilli : Bacillus is catalase positive where Clostridium is catalase negative
-
- All enterobacterales (a gram negative bacilli) are catalase positive
 - Listeria monocytogenes (a gram positive bacilli) are catalase positive

How to do the test

1. Slide method

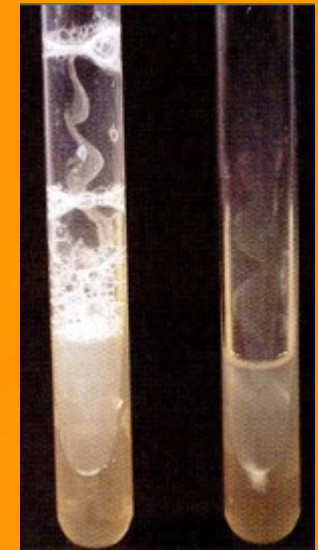
by adding a drop of hydrogen peroxide on a slide and mixing it with a loop of bacteria



2. Capillary tube method

By taking hydrogen peroxide in capillary tube and applying it to the colony on petri-dish directly

3. Adding hydrogen peroxide directly to a pure slant culture



Notes

- Be careful when using bacteria from blood agar culture and avoid touching the agar by the loop because blood cell in agar also had catalase enzyme. → false positive
- Also don't use bacteria from old culture because the enzymes activity drops by time. → false negative