KING ABDULAZIZ UNIVERSITY

Microbial Enzymes II

Physiology of microbiology
Bio 336

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen (such as bacteria,plants, and animals) which catalyzes the decomposition of hydrogen peroxide to water and oxygen.

It is a very important enzyme
in protecting the cell from oxidative damage by reactive oxygen species (ROS).

1- Catalase Activity

Hydrogen peroxide (H2O2) is a poisonous byproduct of metabolism that can damage cells if it is not removed. All respiring organisms therefore must have some mechanism for detoxification. Catalase is one of the common methods. When hydrogen peroxide is added to a colony of catalase-producing bacteria, it is broken down into water(H2O) and oxygen gas (O2). that is produced can be seen as bubbles.

$$2H_2O_2 \text{--------catalase----------->} 2H_2O + O_2$$

Experiment # 1

Catalase activety test

Purpose: Identify the presence of the enzyme Catalase

- Material and Experimental Protocols:
- Observation and results:
- Conclusion:

Microbial Enzymes II

Catalase production test

2- Oxidase activity

Oxidase is an oxidizing enzyme within the series of respiratory enzymes responsible for oxidative phosphorylation reactions, which can oxidize certain aromatic amines such as Phenylenediamine to form end-color products.

If bacteria is positive the paper will turn purple.

A blue- purple color produced is indicative of a positive test.

Phenylenediamine $\xrightarrow{\text{Oxidase}}$ perpul color

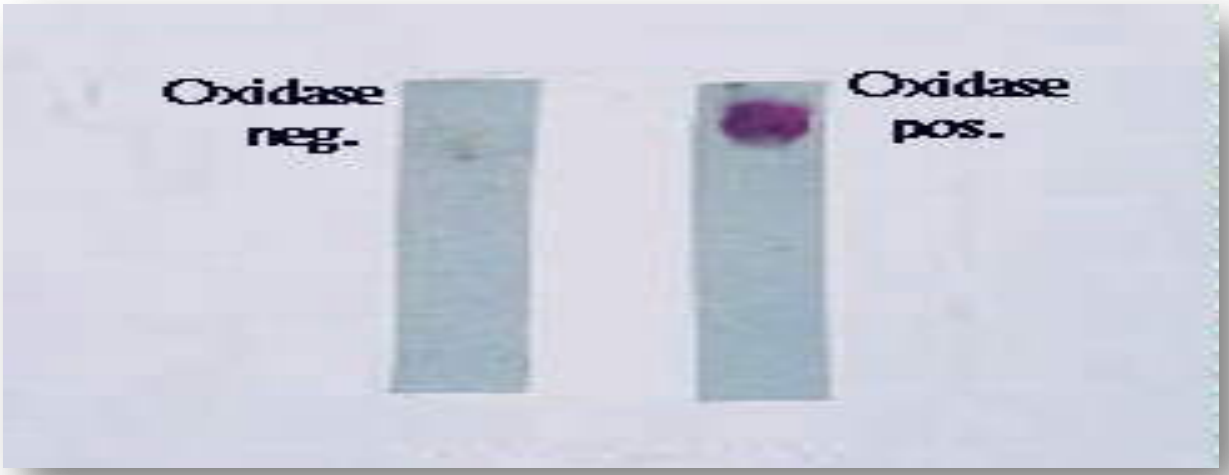
This test is used to identify microorganisms containing the enzyme
cytochrome oxidase (important in the electron transport chain).
It is commonly used to distinguish between oxidase negative *Enterobacteriaceae* and oxidase positive *Pseudomadaceae*.
Cytochrome oxidase transfers electrons from the electron transport chain
to oxygen (the final electron acceptor) and reduces it to water.
In the oxidase test, artificial electron donors and acceptors are provided.
When the electron donor is oxidized by cytochrome oxidase it turns a dark purple.
This is considered a positive result. In the picture below the organism on the right (*Pseudomonas aeruginosa*) is oxidase positive.

Experiment # 2

Oxidase activity test

Purpose: Identify the presence of the enzyme Oxidase

- Material and Experimental Protocols:
- Observation and results:
- Conclusion:



Oxidase activity test

3- hydrolysis of Cellulose

Cellulose is a complex carbohydrate that is composed of many sugars. Some bacteria can enzymatically dissolve cellulose under aerobic conditions to glucose units. This activity is of great importance in the decomposition of plant residues in soil where microorganisms do not accumulate in the soil.

There are two types of cellulose decomposition:

1 - Aerobic decomposition (Debous media).

2 -Anaerobic degradation(Omlyanisky media)+oil.

Experiment # 3

Aerobic decomposition of cellulose

Purpose: To determine whether aerobic bacterium produces a cellulases that will hydrolyze a cellulose .

Dubos medium

- Material and Experimental Protocols:
- Observation and results:
- Conclusion:

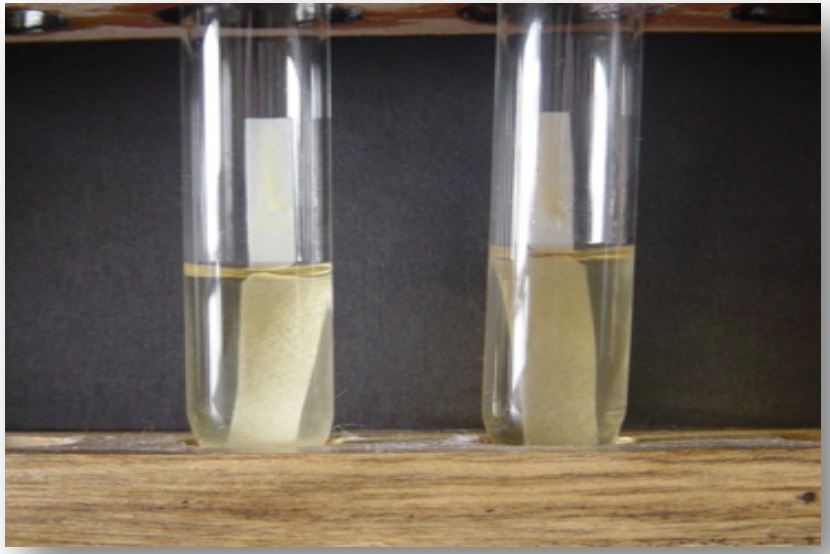
Experiment # 4

Anaerobic decomposition of cellulose

Purpose: To determine whether anaerobic bacterium produces a cellulases that will hydrolyze a cellulose .

Omliansky's medium

- Material and Experimental Protocols:
- Observation and results:
- Conclusion:



Cellulolysis

Catalase & oxidase activity

http://www.uwyo.edu/molb2021/virtual-edge/lab10/lab10_rapid.html
https://www.youtube.com/watch?v=7bkXl_iT6lQ&t=59s

https://www.youtube.com/watch?v=PGBf7Xehcns

https://www.youtube.com/watch?v=brBgR589KFo

https://www.youtube.com/watch?v=z1HsdAQO7m4

https://www.youtube.com/watch?v=YSPbMdR2K70

hydrolysis of Cellulose

https://www.khanacademy.org/science/ap-biology/chemistry-of-life/introduction-to-biological-macromolecules/v/hydrolysis