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Catalase Test: A Biochemical Protocol for Bacterial Identification

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SUMMARY

The catalase test helps the detection of the enzyme catalase in bacteria. It is essential for separating catalase-positive Micrococcaceae from catalase negative *Streptococcaceae*. While it is actually useful in separating between genera, it is also important in speciation of certain gram positives such as *Aerococcus* urinae which is gram positive from *Aerococcus* viridians which is gram negative. Catalase was first observed in 1818 by Louis Jacques Thénard, who discovered (H_2O_2).

INTRODUCTION

The catalase test is a biochemical test for aerobic organisms that enable us to notice the production of catalase enzymes in the microbes. This Catalase enzyme mainly survives in oxygen and is the most common enzyme that is found in all living organisms that mainly survive in oxygen and catalyses the breakdown of hydrogen peroxide, releasing water and oxygen. The catalase test is useful in the theoretical characterization of most bacteria. Under aerobic conditions, 3% H2O2 is used, whereas 15% H2O2 is used in case of anaerobic conditions. This Catalase enzyme defends the organism from oxidative damage from the reactive oxygen species. The enzyme reduces the bactericidal effects of hydrogen peroxide, and its concentration in bacteria has been matched with the pathogenicity of the organism. The catalase test helps in the separation of catalase-positive organisms like *staphylococci* from catalase-negative species like streptococci.

Objectives of Catalase Test: To find out whether the organism is catalase-positive organisms like micrococci and *staphylococci* from catalase-negative organisms like *streptococci* based on the production of catalase enzyme.

Principle of catalase test: The aerobic and facultative anaerobic microorganism's metabolites give rise to toxic by-products like hydrogen peroxide and superoxide radical (O2). These are considered toxic to the organisms and can even result in lysis of cells if not broken down. In the case of pathogenic organisms, they present different mechanisms that break down these products to non-toxic substances. These organisms produce enzymes namely catalase hydrolysis that helps in breaking down hydrogen peroxide into water and gaseous oxygen, that can be confirmed by production of gas bubbles.
The mechanism is given below:

$$H2O2 \xrightarrow[\text{catalase}]{} H2O + O2$$

The main aim of producing catalase enzymes by the organism is to protect itself against the lethal effect of hydrogen peroxide gathering at the end of the aerobic metabolism. To confirm the presence of the catalase enzyme we need to add hydrogen peroxide to the bacterial inoculum, if an oxygen bubble produces it means catalase is present and no bubble means absence of enzyme.

Reagents and Supplies Used: Hydrogen peroxide reagent,30% H2O2 for Neisseria 15% H2O2 for anaerobes, 3% H2O2 for other bacteria, Glass slide, Sterile wooden or glass sticks.

Procedure of Catalase Test:

Many methods are available for catalase tests methods like

1. Slide or drop catalase test,
2. Tube method,
3. Heat-stable catalase mainly used for the separation of *Mycobacterium* species. The semi quantitative catalase for the finding of *Mycobacterium tuberculosis*. the protocol of slide methods is given below

- First a microscope slide needs to be placed in a petri dish. The petri dish is used to limit catalase aerosols, which may take viable bacterial cells.
- Then a small amount of organism is collected from a well-isolated 18- to 24- hour colony with a sterile inoculating loop and placed onto the microscope slide.

- Later add a drop of 3% H2O2 on the microscope slide containing our organism by using a dropper or Pasteur pipette.
- Then we observe bubbles against a dark background to enhance readability.

Result and Interpretation of Catalase Test:

The positive test is confirmed by the immediate formation of bubbles. The appearance of a few bubbles tells of a weak reaction. A negative test is represented by no bubbles or a few bubbles after 20 s.

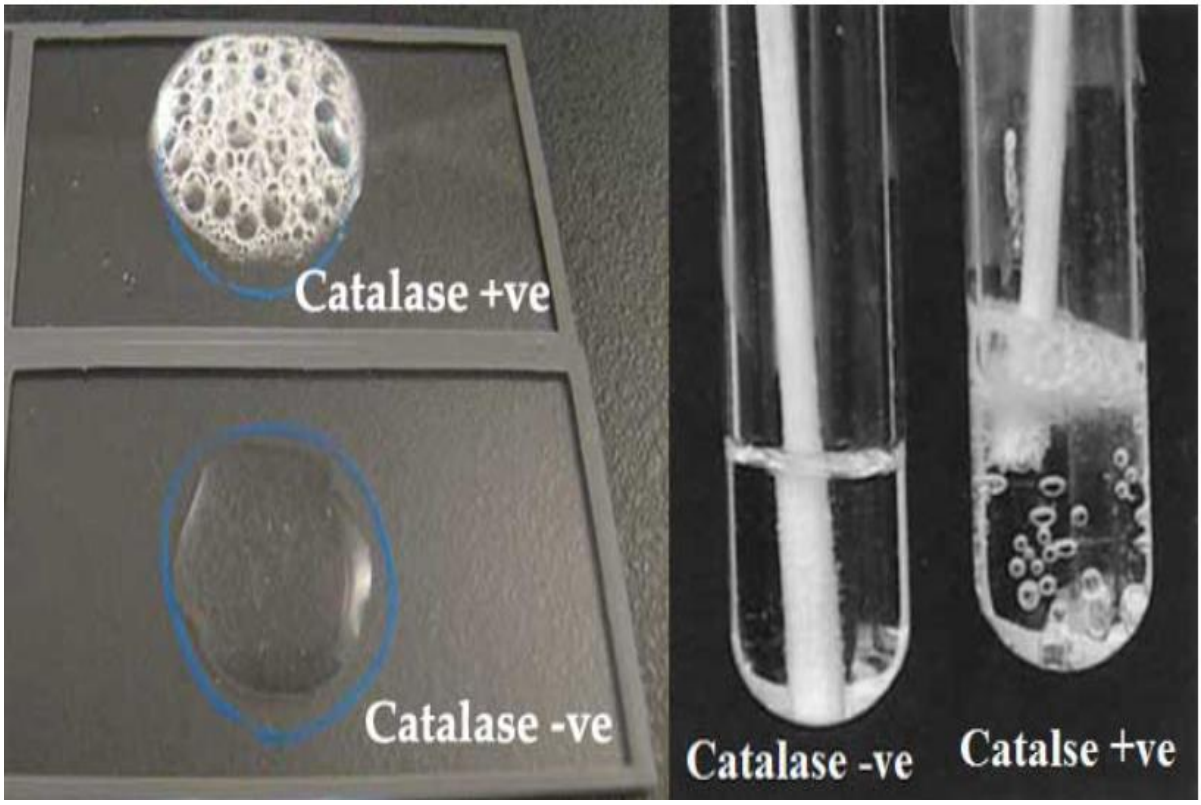

Fig: Result Interpretation of Catalase Test (**Source:** microbiology info.com)

Result: In the result of the catalase test it has been found to separate between *staphylococci* which is gram positive from *streptococci* and enterococci which is gram negative and *Bacillus* which is catalase-positive from catalase-negative *Clostridium* spp.

Applications of catalase test

- Catalase test also allows separating aerobic and obligate anaerobic organisms.
- This is an essential test for the separation of aero-tolerant catalase-negative strains of *Clostridium*, from catalase positive Bacillus.
- This catalase test is important in the speciation of certain gram-positive organisms that has been used to separate between genera.

Limitations of catalase test

- Using metal loops while collecting colonies might yield false-positive results; using platinum loops does not yield false-positive results.
- Since the enzyme is present only in viable cells, the older cultures may give false-negative results, colonies that are older than 24 hours should not be used...
- The reagent and the colony should not be intermixed.
- The reagent can be toxic to humans like 30% H2O2 is extremely destructive to the skin. If contact occurs, wash instantly with 70% ethyl alcohol, not water

CONCLUSION

The catalyse tests are used to find out the presence of catalase, which is an enzyme that catalyse the toxic substance hydrogen peroxide into water and oxygen. A positive result can be made out by the presence of a bubble. Absence of bubbles confirms, it is a negative result; and ensures that the organism does not produce catalase.

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