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Catalase test

Catalase is a haem (heme) containing enzyme whose role is to degrade hydrogen peroxide (H_2O_2). It exists as tetramer with each subunit containing a haem group in the active site. Oxidation of flavoproteins results in the production of Reactive Oxygen Species (ROS) such as superoxide and hydrogen peroxide, which are toxic to bacterial cells. Superoxide ion (O_2-) is produced during oxidative metabolism of the aerobic bacteria, which is removed by the enzyme superoxide dismutase.

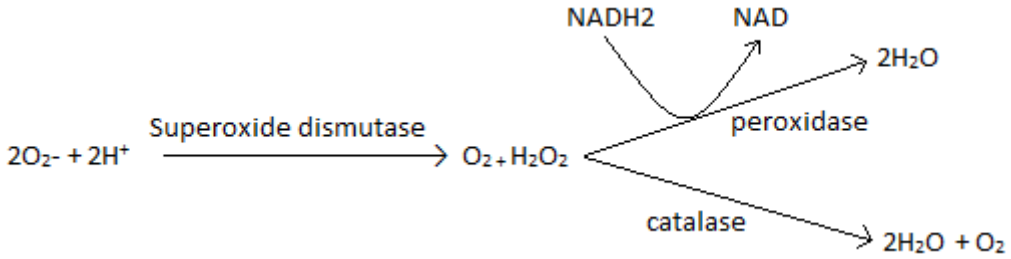
$2O_2^- + 2H^+ \text{-----} \rightarrow H_2O_2 + O_2$

However, this process creates another highly reactive product, the H_2O_2, which is decomposed by the enzyme catalase.

$2\ H_2O_2 \text{-----} \rightarrow 2H_2O\ + O_2$

Anaerobic bacteria lack catalase, but they decompose H_2O_2 with the help of enzyme peroxidase. These enzymes derive electrons from NADH2 and reduce H_2O_2 to H_2O.

$NADH + H^+ + H_2O_2 \text{-----} \rightarrow NAD^+ + 2H_2O$



Types of catalase:

Three classes of catalase are known to occur among bacteria: monofunctional catalases, catalase-peroxidases, and manganese catalases (pseudocatalases). Haem exists as a prosthetic group in the monofunctional catalases and the catalase-peroxidases.

Catalase-peroxidases are also haem containing enzymes that exhibit both catalase and peroxidase activity. They are dimers of two identical subunits and are different from the heam-containing catalases. These types occur among different types of bacteria, including Enterobacteria. In some isolates they are produced along with other catalases.

Non-haem manganese catalases contain a binuclear manganese complex as their catalytic active site instead of haem. These were initially observed in anerobic bacteria (Lactobacillus), which produced neither haem nor cytochrome. These have been noted in *Lactobacillus plantarum*, *Thermus thermophilus*, *Desulfobacterium spp*, *Clostridium spp, Pseudomonas aeruginosa, Salmonella typhimurium* etc.

Enterococcus faecalis are generally catalase negative; they don't synthesize haem or porphyrin. However, when grown in haem containing medium, they can synthesize hemoproteins. Some strains are known to

give a weakly positive catalase test, which is attributed to the presence of some non-haem catalase (pseudocatalase). *Enterococcus haemoperoxidus* is known to give a positive catalase reaction when cultivated on blood agar, but not when grown on blood-free medium. Some strains of *E. fecalis* too give such a reaction.

Catalase test:

When cells of bacteria known to produce catalase are exposed to a solution of 3% hydrogen peroxide, the oxygen released following decomposition of H_2O_2 appears as effervescence or bubbles. Catalase test can be done on slide, tube or agar plate.

Slide catalase test: a small portion of young bacterial colony is transferred from culture plate to the surface of clean and dry glass slide using a straight wire. A drop of 3% H_2O_2 is placed on the colony. Production of effervescence (bubbles) in 5-10 seconds is a positive test. No appearance of bubbles or appearance after 30 seconds (due to degradation of H_2O_2 by other enzymes) is considered negative.

Tube catalase test: Approximately 2-3 ml of freshly prepared 3% H_2O_2 is taken in a test tube. A portion of the young bacterial colony is picked up using a sterile glass capillary, plastic or wooden stick and immersed into the tube containing H_2O_2. Production of effervescence (bubbles) in 5-10 seconds is a positive test.

Plate catalase test: One drop of freshly prepared 3% H_2O_2 is carefully placed over a single colony with the help of a dropper. The lid of the plate must be closed immediately. Production of effervescence (bubbles) in 5-10 seconds is a positive test. This method must not be performed on blood agar plates.

Positive control: *Staphylococcus aureus* ATCC 33592

Negative control: *Enterococcus fecalis* ATCC 29212

Precautions:

- Nichrome wire or loop must not be used to pick colonies as these can give false positive reactions.
- Picking colonies from blood agar must be avoided. If RBCs from the medium gets scraped along with the bacteria, they may give false positive reactions.
- 3% H_2O_2 must be freshly prepared and must be held in dark or amber coloured bottles (protected from light).
- Commercially, H_2O_2 is sold as 100 volumes (30%), which must be diluted to 10 volumes (3%) before use. H_2O_2 is diluted in distilled water.
- Pseudocatalase reaction can be seen in bacteria grown in media with low levels or no glucose. This can be overcome by growing them on media with at least 1% glucose.
- When performing the test on slide or agar plates, one must cover the test with a petri plate to avoid getting exposed to aerosols that might be released.

Variations of catalase test:

For certain anaerobic bacteria, 15% H_2O_2 is used instead of 3%. Anaerobic cultures should be exposed to ambient air for at least 30 minutes before testing and the colonies may be 24-72 hours old. For this test, *Bacteroides fragilis* ATCC 25285 and *Clostridium perfringens* ATCC 13124 are used as positive and negative controls, respectively.

Superoxol test uses 30% H_2O_2 instead of the regular 3% H_2O_2. This test is useful in *differentiating Neisseria gonorrheoae* from other Neisseria species. Most gonococci are positive whereas most meningococci and other Neisseria species are negative or weakly positive. *Neisseria gonorrhoeae* ATCC 19424 and *Neisseria meningitidis* ATCC 13090 are positive and negative controls, respectively.

S**emiquantitative catalase test** is used in the identification of Mycobacteria. Mycobacterial growth (in liquid medium or colonies) is inoculated in LJ medium and incubated for two weeks at 35^oC in an atmosphere of 8-10% CO_2 with loosened caps. To this, 0.5 ml catalase (containing 1:1 ratio of 10% polysorbate 80 and 30% H_2O_2) reagent is added and cap tightened. Cultures of positive (*M. flavescens* ATCC 14474) and negative controls (*M. tuberculosis*) must be test simultaneously. The tubes are allowed to stand at room temperature for five minutes and the column of effervescence above the medium surface is measured. *M. kansasii, M. simiae*, most scotochromogens, nonphotochromogens and rapid growers usually produce a column of more than 45 mm whereas *M. tuberculosis, M. marinum, M. avium complex, M. xenopi* produce column less than 45 mm. Isolates of *M. tuberculosis* (ATCC 35825) that are isoniazid resistant (due to mutation the *KatG* gene) are negative for this test.

Thermocatalase test is a variant of catalase test used in the identification of Mycobacteria. The catalase enzymes of *M. tuberculosis* are inactivated at 68°C whereas enzymes of some other species are not. Mycobacterial culture is suspended in 0.5 ml 15 M phosphate buffer (pH 7) and heated to 68°C for 20 minutes in a water bath. After cooling, 0.5 ml of 1:1 Tween80-30% H_2O_2 reagent is added and let to stand at room temperature (for 20 minutes) and observed. Formation of bubbles is a positive test. *M. tuberculosis* ATCC 15177 and *M. fortuitum* ATCC 6841 are negative and positive controls, respectively.

Alternative tests:

Benzidine test: This test detects iron-porphyrin systems of the aerobic respiration in catalase- and cytochrome-containing bacteria. In the presence of iron-porphyrin compounds and hydrogen peroxide, benzidine forms quinoidic bonds giving a blue color to the reaction mixture. The test is positive for all members of the Micrococcaceae including those which are catalase negative. The test organism is cultured on a non-blood medium (such as Tryptone glucose yeast agar) and the plate is flooded with benzidine dihydrochloride solution followed by equal volumes of 5% H_2O_2 reagent. If the bacteria possess iron-porphyrin compounds, colonies develop blue-green to deep blue color.

31st January, 2017