HELSINGIN YLIOPISTO
MAATALOUS-METSÄTIETEELLINEN TIEDEKUNTA

OPETTAJIEN AKATEMIA

Decomposing hydrogen peroxide with a catalase enzyme

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Objectives

The objective of this exercise is to test/study the ability of aerobic microbes to break down the hydrogen peroxide formed during metabolism with the catalase enzyme.

Background

Catalase is a common enzyme found in microbes and animals. Catalase decomposes hydrogen peroxide (H_2O_2). Hydrogen peroxide is formed in low quantities in the metabolism of all aerobic organisms. Hydrogen peroxide is toxic because of its strong oxidation capacity. In the cell, catalases function by breaking down hydrogen peroxide into oxygen and water. In humans, the catalase enzyme is formed mostly in the liver and kidneys.

The catalase test is used as one of the biochemical tests when grouping bacteria. Based on the results, bacteria are divided into catalase positive or catalase negative groups. Many of the catalase negative bacteria live in anaerobic conditions and no hydrogen peroxide is formed during metabolism. Lactic acid bacteria that grow in aerobic environment are also catalase negative, but in them the hydrogen peroxidase is broken down with the peroxidase enzyme. Peroxidase uses the oxygen in the hydrogen peroxide as a substrate when oxidizing other compounds.

Principles

The purpose of this exercise is to test/study the ability of aerobic microbes to break down hydrogen peroxide with the catalase enzyme. The exercise is performed by cultivating different microbes on solid growth media. Microbial mass is harvested from colonies and transferred on to a microscope slide. A couple of drops of 3 % hydrogen peroxide are added to the microbial mass. The oxygen released from the microbes makes the hydrogen peroxide froth in the catalase positive microbes. No reaction is observed in the catalase negative microbes.

Lab safety

The microbes used are not harmful to humans, but they should nonetheless be handled with care and contamination should be avoided. It is recommended that gloves are worn when

working with microbes. The 3 % hydrogen peroxide may irritate the skin and eyes, splashes need to be rinsed with running water.

Time required

15–30 min or 1-2 days, if the microbes are not cultivated beforehand

Equipment

- 3 % hydrogen peroxide, store in the fridge as the effect weakens with time
- Pure microbial cultures:
  - *Escherichia coli, Bacillus subtilis* (catalase positive)
  - *Lactococcus lactis, Streptococcus* sp. (catalase negative)
- Solid growth media set in Petri dishes, such as TY or TGY. Disposable Petri dishes and media can be purchased from VWR International. You can also enquire after ready-made plates from Mika Kalsi at the Department of Food and Environmental Sciences in the Faculty of Agriculture and Forestry (University of Helsinki), mika.kalsi@helsinki.fi
- Sterile glass rods or transfer loops
- Disposable Pasteur pipets
- Gloves
- Microscope slide or equivalent for transferring the microbe mass on to

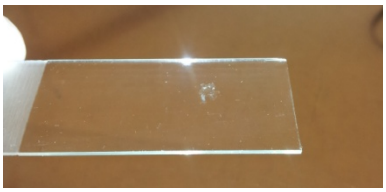

Figure 1: Microbial mass transferred on to a microscope slide.

Instructions

If the exercise is done with plates cultivated beforehand, start from #2.

1. Transfer one microbial strain per plate / petri dish (on for example TGY agar). Each plate should be marked well. The microbes are grown for 1-2 days in a temperature suitable for each microbe.

2. Transfer some of the microbe mass with a wooden rod on to a microscope slide (figure 1).

3. Add a drop of the hydrogen peroxide on to the microbe mass (figure 2). A positive reaction can be observed as frothing of the hydrogen peroxide (figure 3).

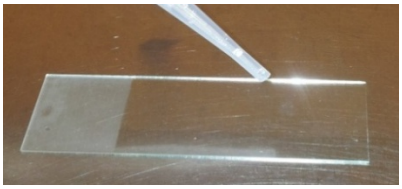

Figure 2: Adding the hydrogen peroxide

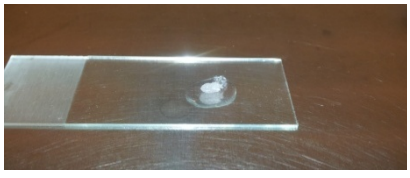

Figure 3: The hydrogen peroxide froths, a sign of a positive reaction.

Observations

- Was there a difference between the catalase positive enzymes in how (much) the hydrogen peroxide frothed?