ISOLATION AND IDENTIFICATION OF INVERTASE ENZYME FROM TOOTH DECAY SAMPLE

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Abstract: The present study was aimed at isolating and identifying Invertase enzyme from bacteria of tooth decay samples. Different decayed tooth samples are collected from dental clinics, they are inoculated into nutrient broth having sucrose as main substrate because invertase present acts on the substrate and releases reducing sugars which are assayed for detection of enzyme activity. Tooth decay is caused by streptococcus mutans which is identified by the staining in present study.
The samples are grown for 24hrs in sucrose presence at 25°c incubation temperature. For enzyme recovery fresh media is inoculated with 0.1 ml of 24hr culture incubated for 8hrs,after 8 hrs culture media is centrifuged at 33000g rpm. The supernatant collected and enzyme assay is carried out by the detection of reducing sugar presence. The action of enzyme on the substrate is calculated by performing quantitative benedicts test

Keywords: invertase, streptococcus mutans, benedicts quantitative reagent, enzyme recovery

Introduction: Enzymes are catalysts which are neither consumed nor permanently altered as a consequence of their participation in a reaction.
Enzymes are extremely selective classified for the type of reaction catalyzed and for a single substrate. Enzymes are stereo specific and convert nonchiral to chiral.
Enzymes are assayed by ELISA and spectrophotometrically. They play important role in causing certain diseases. For some diseases excessive activity of enzyme may be a cause.
Measurements of the activity of enzymes in blood plasma, erythrocytes or tissue samples results in diagnosing certain illness. The ability of enzyme to rapidly transform thousands of molecules of a specific substrates into products imbues each enzyme with the ability to reveal its presence.
Assays of the catalytic activity of the enzymes are frequently used in research and clinical laboratories. Under appropriate conditions the rate of the catalytic reaction being monitored is proportional to the amount of

enzyme present which allows its concentration to be inferred.

Enzymes lacking catalytic activity are assayed by ELISA technique. Enzyme producing microorganisms are isolated by the substrate addition in the media for their growth. Difference in the growth of microorganisms is observed depending on pH and temperature. Microorganisms deposited during different cultures usage are tested for activity by biochemical reactions. During these reactions unexpected findings and discovery may occur.

Materials And Methods: Sample collection: Different decayed tooth samples are collected from different clinics and inoculated into appropriate media. Grown for 24hrs and enzyme sample collected

Media and chemicals preparation:

1) Sucrose media
2) Citrate phosphate buffer
3) Benedict's quantitative reagent
4) 1% sodium hydroxide

Sucrose Media Preparation:

Sucrose -10g/dl
Yeast extract-0.5g/dl
Tryptone-0.5g/dl
Dipotassium hydrogen phosphate-0.1g/dl

10 gm of sucrose ,0.5gm of yeast extract 0.5 gm of tryptone ando.1gmof dipotassium hydrogen phosphate are weighed accurately and dissolved in 100ml of distilled water,the media is sterilized in autoclave at 121°cfor 15min at 15lbs pressure.after sterilization the media is cooled to room temperature .

Citrate phosphate buffer: 0.2M, PH 4.5: Buffer is prepared by mixing 24ml of citric acid solution with 100ml of disodium hydrogen phosphate.

1% Sodium Hydroxide: 1gm of sodium hydroxide dissolved in 100ml of water and 1% sodium hydroxide is prepared before performing experiment.

Method: After sterilization and cooling the media is inoculated with sample and incubated for 24hrs at 25°c. culture obtained after 24 hrs is collected .Culture collected is again inoculated into fresh media on second day for 8hrs at 25°c for enzyme recovery.

Enzyme Recovery: Enzyme breaks down sucrose to give fructose and glucose. Amount of glucose released is measured by the benedict's

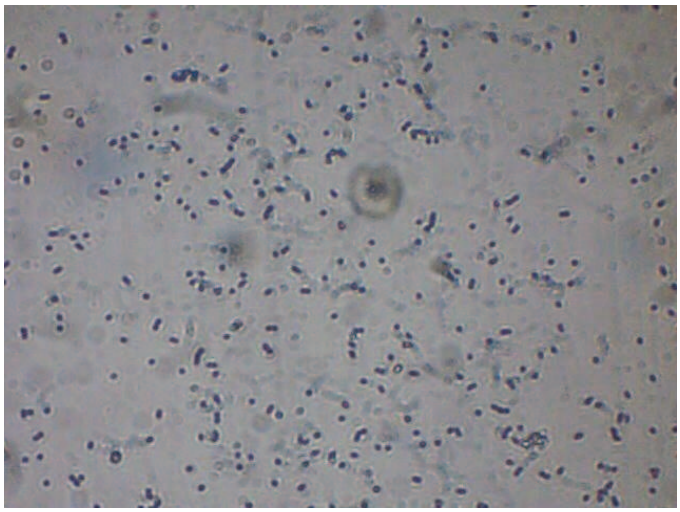
quantitative method.0.3M Sucrose is used as substrate .A liquots are made to a total volume of 2.5ml and buffered with 1.5ml of 0.2M Citrate phosphate buffer pH 4.5.To this 1ml of enzyme solution is added and the mixture is incubated at room temperature for 15min.The reaction is stopped by adding 0.5ml of 1% sodium hydroxide and after removing any sediments from the supernatant glucose is estimated by benedict reagent from the solution available after centrifugation

Benedict's reaction: 2.5ml of 0.3M Sucrose is mixed with 1.5ml of 0.2M Citrate phosphate buffer.To this 1ml of enzyme solution is added and incubated at 37°c for 15min.To this 0.5ml of 1% sodium hydroxide is added and sediments are removed. Estimation is carried out by titrating enzyme solution with 10ml of benedict's reagent, 20ml of water and 5gm of sodium carbonate.

Results and discussion: According to standard equivalence factor of benedict reagent 10ml of benedict reagent require 20mg of glucose,So the in above research work 10 ml of benedict consumed 18ml of enzyme test solution So from the standard calculation 20/18=1.6

1.6mg of glucose is released from the substrate by the enzyme invertase action.

The culture is tested for the micro organism identification by gram staining.



Conclusion and findings: Different tooth decay samples are studied for the enzyme production. All the samples produce different sucrolytic

enzymes. Invertase produced is studied by its isolation and detection with quantitative Benedict reagent .The samples are reported to contain organism streptococcus mutans by different staining studies.The mentioned organism is responsible for tooth decay revealed from referred research work.

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9. Scandavian Journal of dental research 1977 Jan-Feb;85(2):101-5.sucrolytic enzymes from dental plaque In saliva

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