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Isolation of Glucan Sucrase Enzyme from Tooth Decay Samples

Phanideepika Polampalli *, Vijay Kumar G[1], and Lakshmi Narasu M[2]

Care College of Pharmacy, Oglapur, Warangal, Andhra Pradesh, India.

ABSTRACT

This study was aimed at isolating and identifying glucan sucrase enzyme from bacterial strains in tooth decay of dental caries patient. Different tooth decay samples are collected and incubated at 37°c for 24 hrs in nutrient agar medium. Culture grown is separated and isolated by streaking. The colonies are collected in broth and centrifuged for extracellular enzyme extraction. The enzyme is assayed by amount of glucose released which is detected by benedict's reagent reduction.

Keywords: Glucan sucrase, dental caries, serial dilution

Corresponding author

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INTRODUCTION

Enzymes from different tooth decay samples constitute amylase, glucan sucrase. Bacteria present in mouth like lactobacillus and streptocoocus species produces glucan sucrase enzyme. This enzyme is responsible for conversion of sugar from food and produces long chain glucose molecules which adhere to the teeth enamel [1]. Glucan sucrose called as glucosyl transferase (E.C. 2.4.1.5) which synthesizes a unique soluble glucan polymer with (1→4) &(1→6) linkages.Two reaction are catalyzed by this enzyme [1]. Hydrolysis in which water is acceptor [2] glucosyl transfer carries polymerization and oligosaccharide synthesis.Glucan sucrase enzyme hydrolyses sucrose into glucose and fructose. Once bacteria ferment sugar releases acids dissolving calcium in teeth causing caries. Glucansucrase enzyme is abundantly present in tooth decay samples.

MATERIALS AND METHODS

Sample collection

Different tooth decay samples are collected and diluted by serial dilution technique. 1gm of sample is weighed and transferred to 10 ml of distilled water. This solution is maintained as stock. From this stock, different dilutions are prepared

Sample isolate preparation

0.1 ml is transferred to the test tube containing 10 ml of distilled water and labeled as 10^{-1}. From this concentration 10^{-9} concencentrations prepared serially.From all the concentrations 0.1 ml of sample is transferred to the nutrient agar petri dishes and slants.For each dilution triplicate plates are prepared these petri plates are incubated for 48 hrs.

Enzyme detection is carried by biochemical test. Colonies from the isolated plates and slants are selected.0.1 ml of theinoculum is inoculated into nutrient broth containing sucrose. The enzyme present in the colonies hydrolyses the polymer into glucose and fructose. [4] Detection of glucose from the broth: glucose liberated from the broth by the action of enzyme is detected by benedict's reagent. Five drops of broth is added to the benedict's reagent kept in water bath. Colour changeof reddish brown is observed.

REPORT: Broth has shown colour change which indicates positive reaction. This indicates glucan sucrase enzyme presence.

RESULTS AND DISCUSSION

The enzyme from tooth decay sample is isolated and detected by the product released from substrate sucrose. Glucose is released from substrate and detected bybenedict's reagent. Increasing volume addition of broth to the reagent has shown colour change in test tube. Benedict's reagent with sodium carbonate and sodium citrate and copper sulphate get reduced

to hydroxide form.Copper oxides in the presence of reducing substances get reduced and change from black to rust brown colour.

CONCLUSION

Glucan sucrase enzyme acts on polysaccharide and hydrolyses the sucrose to the monosaccharide glucose and fructose.The enzyme activity is studied by its action the substrate and the product released.

Different samples are grown on sucrose containing media and colonies isolated and grown in nutrient broth.This broth is added in increasing volumes to the reagent which shows variable sensitivity.

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