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PRODUCTION AND OPTIMIZATION OF DEXTRANASE ENZYME FROM *STREPTOCOCCUS MUTANS* CAUSING DENTAL PLAQUES

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ABSTRACT

S. mutans isolated from the human dental plaques was screened for the presence of dextranase enzyme. Various optimization parameters like temperature, moisture content, incubation days, pH, carbon and nitrogen sources were analyzed under anaerobic conditions for maximum dextranase enzyme production by the submerged fermentation technique with dextran as the substrate. Dextranase production was optimum at a temperature of 37°C, with 50% moisture content in 3 days with sucrose as the carbon source and yeast extract as the nitrogen source, at pH 5.5.

Keywords: *S. mutans*, dextranase enzyme, submerged fermentation.

INTRODUCTION

The etiology of dental caries is often associated with the increasing amounts of various acidogenic microorganisms like *Streptococcus mutans* which plays a key role in the formation of cariogenic biofilms [24]. *S. mutans* is the primary odontopathogen present in supragingival plaque and causes the oral disease [3]. The organism utilize sucrose for the production of sticky, extracellular dextran by the enzyme dextransucrase [20]. Dextran is a homoglycan of α-D-glucopyranose molecules coupled primarily with α-1, 6 linkages. It is a component of dental plaque, which is considered to contribute to the development of dental caries [14].

Dextrans are degraded by dextran- hyrdolyzing enzyme, dextranase. Dextranase [1, 6 α-D-glucanohydrolase, EC 3.2.1.11] has an important industrial applications since the enzyme

depolymerize various troublesome microbial dextran deposits in teeth and prevents toothdecay. The enzyme have a high substrate specificity and is isolated from a wide range of microorganisms [14]. It is briefly classified into exo and endo dextranases [1].

In *S. mutans*, dextran is degraded by dextranase to isomaltosaccharides, predominantly isomaltotriose, isomaltotetraose and isomaltopentaose [7, 11]. The extracellular dextranase partially degrade the glucans or modify glucans by altering the ratio of 1, 6-α- to l, 3-α-linked chains and hence decrease solubility in water [10] and therefore affect virulence of *S. mutans.* Thus the involvement of dextranase activity in affecting the composition of dental plaques has been evident. The purpose of the study was to optimize the growth conditions of *S. mutans* for the production of dextranase enzyme on various parameters and a preliminary partial report has been given.

MATERIALS AND METHODS

Isolation and identification of *Streptococcus mutans*

The dental plaques from humans were swabbed from the supragingival cavity of the mouth using a swab. The selective media, mitis- salivarius agar (MS) was used for the identification of *S. mutans*. The media was autoclaved at 121°C for 15 min and the plates were incubated for 48 h anaerobically [23].

Optimization under submerged fermentation

Optimization studies of various physiochemical parameters were carried out in a 250 ml Erlenmeyer flask containing tryptic soy (TS) broth for the production of dextranase enzyme [23]. After sterilization by autoclaving, the flasks were cooled and inoculated with *S. mutans* and maintained under various operational conditions separately such as temperature (17, 27, 37, 47 and 57ºC), moisture content (10, 20, 30, 40, 50, 60 and 70%), incubation period (1, 2, 3, 4 and 5 days), pH (1.5, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5), carbon sources (sucrose, glucose, maltose, starch and fructose), nitrogen sources (peptone, yeast extract, casein, urea and albumin) and the culture filtrate were analysed for dextranase production for every 24 h.

Enzyme assay

Dextranase assay was performed by the method of Janson and Porath [13]. The enzyme activity was determined with the DNS (dinitrosalicylic acid) reagent with 1.9 ml of dextran (2% dextran in 0.1M potassium phosphate, pH 6.0) as the substrate. One unit (U) activity of enzyme liberates 1 µmole of isomaltose per min at pH 6 at 37°C. Quantitative estimation of

proteins was determined by the method of Lowry [17] using bovine serum albumin as a standard.

Statistical analysis

The results obtained were expressed as mean ± SD.

RESULTS AND DISCUSSION

Effect of temperature for the dextranase enzyme production

The temperature plays an important role in the production of dextranase and in the determination of the efficiency of dextranase [12]. The highest enzyme production was observed at 37°C with 59.97 U/ ml (Fig. 1).

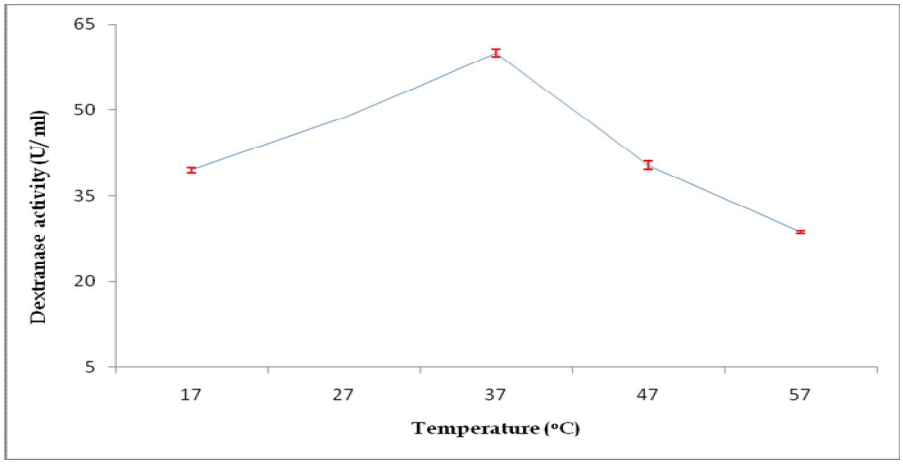

Fig. 1 Effect of temperature on the dextranase enzyme production

The temperature ranges for growth of *S. mutans* was found to be very narrow, from about 30 to 47°C, with optimal growth around 37°C. Thus, the organisms showed little potential to grow in the environment outside the host [18]. Bailey *et al*., [2] reported that the temperature at 37°C was more convenient for the investigation of dextranase enzyme activity.

Effect of moisture content for the dextranase enzyme production

Interactions between an enzyme molecule and the surrounding water are of crucial significance for enzymatic catalysis [15]. In the present study the effective moisture concentration required to produce dextranase enzyme was recorded in *S. mutans* at 50% with 71.44 U/ ml. There was an increase in the enzyme production as the amount of the concentration of water increases and there was a gradual decrease in the enzyme production as the concentration increases after 50% in *S. mutans* (Fig. 2).

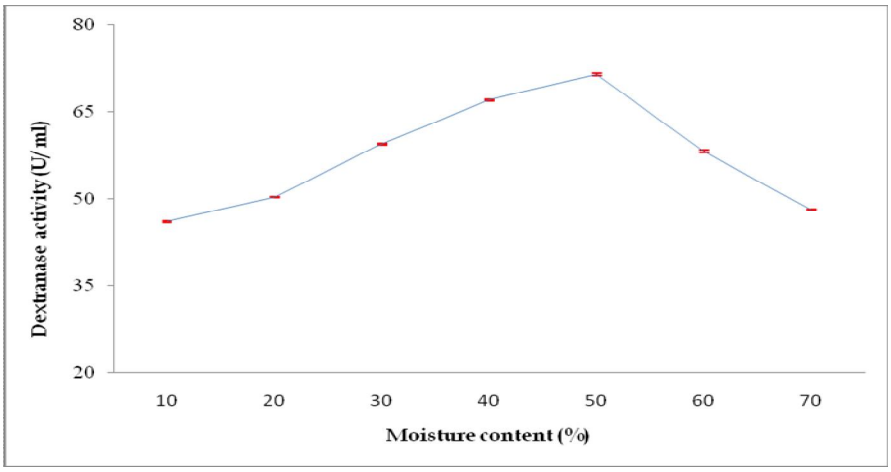

Fig. 2 Effect of moisture content on the dextranase enzyme production

The higher the water content, the greater the enzymatic activity [27]. In earlier reports at the range from 10- 50%, *Bacillus subtilis* NRC-B233b produced maximum amount of dextranase enzyme at the rate 170.6 (U/ g) with 20 (v / w) of moisture [9].

Effect of incubation period on the production of dextranase enzyme

The maximum enzyme production was observed on day 3 with the enzyme activity of 57.9 U/ ml (Fig. 3). Earlier work demonstrated by Staat *et al.*, [22] suggested that the incubation of *S. mutans* to 3 days was sufficient for the production of the dextranase enzyme. Similarly, in the present study the maximum enzyme production was observed on day 3 with the initial graduation from day 1 and slowly the activity of the enzyme decreased during the prolonged incubation of the organism.

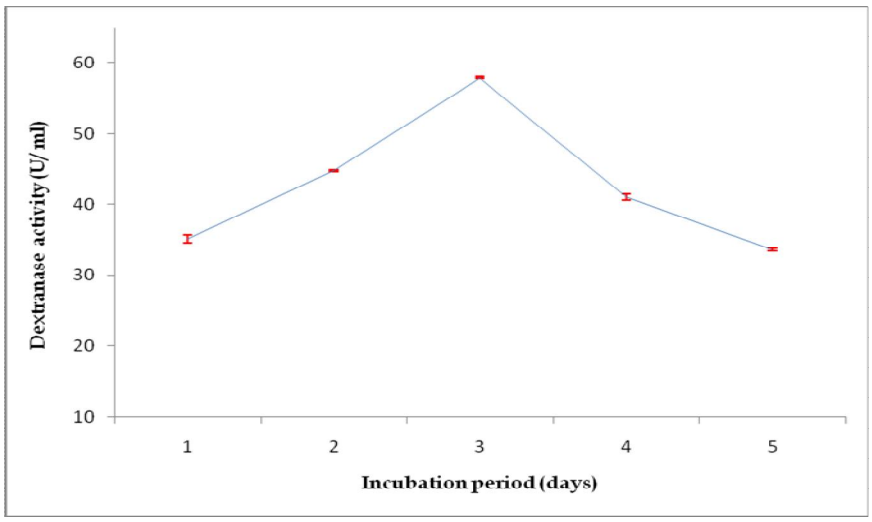

Fig. 3 Effect of incubation days on the production of dextranase enzyme

Effect of pH for the dextranase enzyme production

The ability of the oral bacteria to tolerate acidic environments is of major importance in the ecology of dental plaque [19]. This is directly related to the pathogenesis of dental caries. The maximum production of dextranase enzyme was recorded at pH 5.5 with 66.06 U/ ml of enzyme activity (Fig. 4). Similar results were confirmed by Ellis *et al.*, [8] where *S. mutans* produced dextranase enzyme at an optimum pH of 5.5 at 37°C. The optimum enzyme activity for the organisms ranged from pH 4 - 5.5 [25] due to the rapid and profound change in the physical properties of dextran and negligible above 6.5 [21]. The increase in activity upon progression is due to the change in the state of the enzyme as its isoelectric point was found to be 4.0 [4] and low concentrations of glucose. pH below 4 was not conductive to rapid growth and significantly gave a lower enzyme activity.

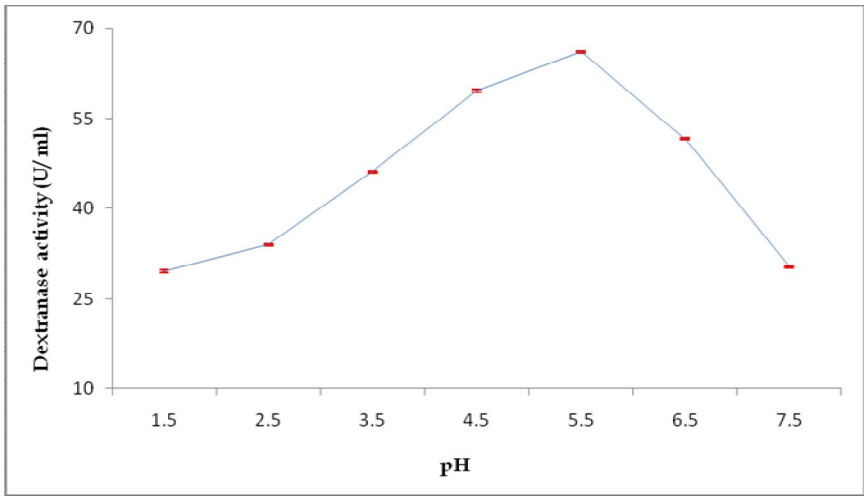

Fig. 4 Effect of pH for the dextranase enzyme production

Effect of carbon sources for the dextranase enzyme production

The cariogenicity of streptococci depends on the sugars as the main energy source [16]. The highest enzyme production of 90.72 U/ ml was observed in sucrose supplemented medium (Fig. 5). Dextranase enzyme was released from *S. mutans* during the exponential phase of the growth and once the maximum activity has reached there was no subsequent fall up to 24 h of inoculation. In the growth medium consisting of sucrose, dextranase released become cell associated because of its affinity for its own substrate and the activity increases throughout the exponential phase finally reaching to its maximum [26]. According to Day *et al.*, [6] the production of the extracellular dextranase has been enhanced by the assimilable carbon

source other than the addition of dextran. Glucose, fructose and sucrose has been preferred as the carbon source for the dextranase enzyme production by *Lipomyces starkeyi*.

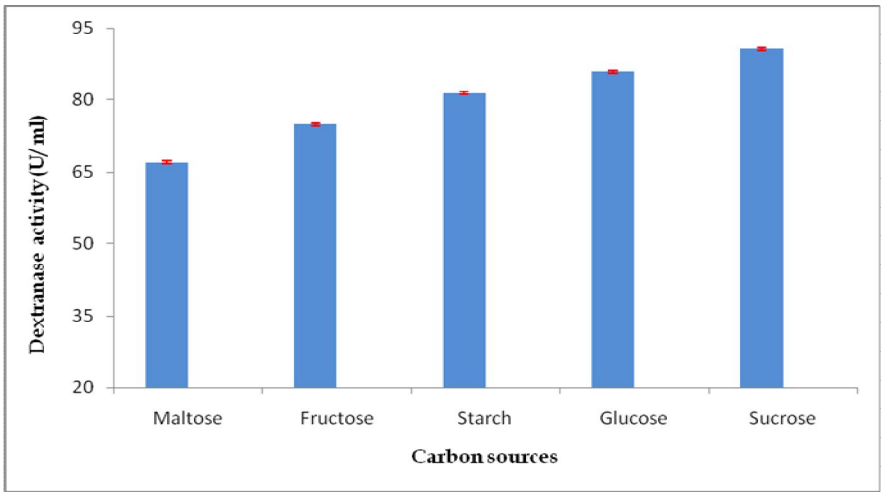

Fig. 5 Effect of carbon sources for the dextranase enzyme production

Effect of nitrogen sources for the dextranase enzyme production

The highest enzyme production was observed in yeast extract with 86.85 U/ ml (Fig. 6). A source of nitrogen is required for the enzyme production. Higher amount of the dextranase production has been possible with the supplementation of nitrogen sources along with the essential minerals and small amount of growth factors [5]. Equal amounts of nitrogen sources and dextran produces a higher level of enzyme. Under the pH controlled conditions neutral pH helps in the greater production. According to Davis *et al.*, [5] corn steep liquor and autolyzed yeast extract were commonly used for the dextranase enzyme production.

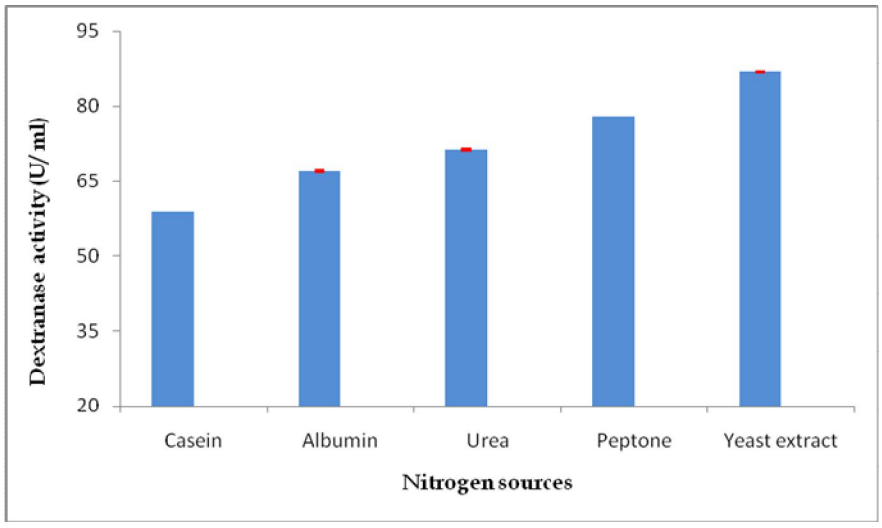

Fig. 6 Effect of nitrogen sources for the dextranase enzyme production

CONCLUSION

The present study indicates that *S. mutans* is capable of producing high amount of dextranase enzyme. The use of submerged fermentation for production of dextranase is an economical process and is very simple to apply. The important parameters had significant positive effects on the dextranase production in different nutrients and cultivation conditions.

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REFERENCES

1. Abdel- Aziz MS, Talkhan FN, Janson J. Purification and characterization of dextranase from a new strain of *Penicillium funiculosum.* J Appl Sci Res, 2007; 3 (11): 1509-1516.
2. Bailey RW, Clarke RTJ. A bacterial dextranase. Biochem, 1959; 72: 49-54.
3. Chatfield CH, Koo H, Quivey RG Jr. The putative autolysin regulator LytR *Streptococcus mutans* plays a role in cell division and is growth-phase regulated. Microbiol, 2005; 151(2): 625-631.
4. Chludzinski AM, Germaine GR, Schachtele CF. Purification and properties of dextransucrase from *Streptococcus mutans.* J Bacteriol, 1974; 118 (1): 1-7.
5. Davis RS, Habra L, Isenberg DL. Method of producing dextranase. US Patent, 1974; 3,787,289.
6. Day DF, Koenig DW. Method of producing dextranase. US patent, 1988; 4,732,854.
7. Dewar MD, Walker GJ. Metabolism of the polysaccharides of human dental plaque. I. Dextranase activity of streptococci and the extracellular polysaccharide synthesized from sucrose. Caries Res, 1975; 9: 21-35.
8. Ellis DW, Miller CH. Extracellular dextran hydrolase from *Streptococcu mutans* (sic *sobrinus*) strain 6715**.** J Dent Res, 1977; 56: 57-69.
9. Esawy MA, Mansouri SH, Ahmed EF, Hassanein NM, El Enshasy HA. Characterization of extracellular dextranase from a novel halophilic *Bacillus subtilis NRC-B233b* a mutagenic honey isolate under solid state fermentation. E J Chem, 2012; 9(3): 1494-1510.
10. Germaine GR, Harlander SK, Leung WLS, Schachtele CF. *Streptococcus mutans* dextransucrase: functioning of primer dextran and endogenous dextranase in water-soluble and water- insoluble glucan synthesis. Infect Immun, 1977; 16: 637-648.

11. Igarashi T, Yamamoto A, Goto N. Characterization of the dextranase purified from *Streptococcus mutans* Ingbritt. Microbiol Immunol, 1992; 36: 969-976.
12. Inkerman PA. An approval of the use of dextranase. Proc Intern Soc Sugarcane Technols, 1980; 17: 2411-2477.
13. Janson JC, Porath J. Methods Enzymol, 1966; 8: 615.
14. Khalikova EF, Susi P, Usanov NG, Korpela T. Microbial dextran- hydrolyzing enzymes: Fundamentals and applications. Microbiol Molbiol Rev, 2005; 69 (2): 306-325.
15. Kuntz ID, Kauzmann W. Hydration of proteins and polypeptides. Adv Protein Chem, 1974; 28: 239-345.
16. Loesche WJ. Dental caries: a treatable infection. Charles C Thomas, publisher, Springfield, Ill, 1982.
17. Lowry OH, Rosebrough NJ, Farr AC and Randall RJ, Protein measurement with Folin phenol reagent, J Biol Chem, 1951; 265-275.
18. Ma Y, Marquis RE. Thermophysiology of *Streptococcus mutans* and related lactic-acid bacteria. Antonie van Leeuwenhoek, 1997; 72(2): 91-100.
19. Nascimento MM, Lemos JAC, Abranches J, Goncalves RB, Burne RA. Adaptive acid tolerance response of *Streptococcus sobrinus*. J Bacteriol, 2004; 186 (19): 6383-6390.
20. Ryan KJ, Ray CG. Sherris Medical Microbiology (4th ed.). McGraw Hill, ISBN 0-8385-8529-9, 2004.
21. Sery TW, Hehre EJ. Degradation of dextran by enzymes of intestinal bacteria. J Bacteriol, 1956; 71: 373-380.
22. Staat RH, Gawronski TH, Schachtele CF. Detection and preliminary studies on dextranase producing microorganisms from human dental plaque**.** Infect Immun, 1973; 8: 1009-1016.
23. Staat RH and Schachtele CF, Evaluation of dextranase production by the cariogenic bacterium *Streptococcus mutans*, Infect Immun, 1974; 9 (2): 467-469.
24. Thurnheer T, van der Ploeg JR, Giertsen F, Guggenheim B. Effects of *Streptococcus mutans* gtfC deficiency on mixed oral biofilms invitro. Caries Res, 2006; 40(2): 163-171.
25. Tsuchiya HM, Jeanes A, Bricker AM, Wilham CA. Dextran- degrading enzymes from molds. 1952; 64: 513-519.
26. Walker GJ, Pulkownik A, Morrey- Jones JG. Metabolism of the polysaccharides of human dental plaque: release of dextranase in batch cultures of *Streptococcus mutans*. J Gen Microbiol, 1981; 127: 201-208.

27. Zaks A, Klibanov AM. Enzymatic catalysis in nonaqueous solvents. J Biol Chem, 1988; 263: 3194-3201.