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PARTIAL PURIFICATION OF DEXTRANASE ENZYME FROM *STREPTOCOCCUS SOBRINUS*

SATHYA G. AND PALANISWAMY M.

Department of Microbiology, School of Life Sciences, Karpagam University, Coimbatore – 641 021, India

ABSTRACT

In the present investigation, the crude dextranase enzyme obtained from *S. sobrinus* was purified by ion- exchange chromatographic technique using DEAE- cellulose. The final purification fold 20.58 and 4.7% recovery was obtained. Molecular weight of 50 kDa was determined by SDS- PAGE.

KEYWORDS: ***S. sobrinus*, dextranase enzyme, DEAE- cellulose**

PALANISWAMY M
Department of Microbiology, School of Life Sciences,
Karpagam University, Coimbatore – 641 021, India

Corresponding author

INTRODUCTION

Dental caries is a microbiologic infection of the tooth which results in localized dissolution and destruction of the calcified tissue [1]. Mutans streptococci colonizing the tooth surface is associated with the etiology and pathogenesis of dental caries in humans and *S. sobrinus* is being frequently isolated from the human dental plaque [2]. The organism grow and carry glycolysis at low pH during the periods of sustained acidification that are conductive to caries development [3]. The organism synthesize extracellular gummy dextrans [4, 5]. Dextrans are high molecular weight glucose polymer joined by the α- 1, 6-glucosidic bonds synthesized from sucrose by the enzyme dextransucrase (E.C. 2.4.1.5) [6]. It has been postulated that such polymers are responsible for the ability of these streptococci to form gelatinous plaques on the surfaces of teeth [4, 5]. The presence of dextran indicates the loss of sugar [7], and as it is a component of dental plaque, which is considered to contribute to the development of dental caries, it has been one of the main driving forces to investigate dextran-hydrolyzing enzymes called dextranase [6]. Dextranase (EC. 3. 2. 1. 11), the extracellular enzyme is of industrially important enzyme owing to its importance in biotechnological application [6]. It removes the dental plaques by degrading the water insoluble glucan synthesized by the dietary sucrose of the organism [8] and eliminates by hydrolyzing the α- l, 6 glucosidic bond of the polysaccharide dextran [6]. The enzyme acts as the clinically potential component in the toothpaste [8]. This paper deals with the purification of dextranase produced by *S. sobrinus* isolated from the human dental plaque.

MATERIALS AND METHODS

Microorganism and growth conditions

The strain *S. sobrinus* used in the study was isolated from dental plaques of humans swabbed from the supragingival cavity of the mouth. The strain was identified using a selective media, mitis- salivarius agar (MS) with aztreonam antibiotic. The flask was autoclaved at 121°C for 15 min, cooled to room temperature and inoculated with 2 mL of bacterial cell suspension which was prepared previously and incubated for 48 h anaerobically [9].

Enzyme production

For the dextranase enzyme production, the organism was inoculated in 250 ml Erlenmeyer flask containing tryptic soy (TS) broth with 1% dextran at 37°C for 48 h anaerobically. The cells were harvested by centrifugation at 10,000 rpm for 10 min. The clear supernatant was used to assay for the enzyme activity [10].

Dextranase enzyme assay

Dextranase assay was performed by the method of Janson and Porath [11]. 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.

Assay of protein content

Quantitative estimation of proteins was determined by the method of Lowry [12] using bovine serum albumin as a standard.

Purification of dextranase enzyme

Crude dextranase from the culture supernatant was precipitated with solid ammonium sulphate at 70% saturation. The precipitate was removed by centrifugation (10,000 x g for 20 min), dissolved in 50 mM sodium acetate buffer (pH 6.0), and extensively dialyzed against the same buffer for 24 h at 4°C [13].

DEAE- cellulose column

After dialysis the sample was loaded to the DEAE- cellulose column (30 × 1 cm) and the elution was carried out at the rate of 2 ml/h equilibrated with the same buffer (pH 6.0). All the purification steps were carried out at 4°C [14].

Determination of molecular weight by SDS-PAGE

SDS-polyacrylamide slab gels were prepared as described by Laemmli [15]. Enzyme proteins (10 µl) was heated for 5 min in 10 mM- Tris/ HCl buffer, pH (8.8), containing 5% (w/v) SDS, 0.25 ml mercaptoethanol. Electrophoresis was performed at 30 mA for 20 h. Proteins were stained with 0.1% silver nitrate solution

RESULTS AND DISCUSSION

Purification of dextranase enzyme

The different steps of purification of *S. sobrinus* is shown in Table 1. Purification with ammonium sulphate was performed to remove other contaminants from the crude extract which gave the purification fold of 3.07 with 47.10% recovery. An additional 12.59 fold resulted after passing the crude to the column of DEAE cellulose I with NaCl (0.1M). The enzyme activity from the chromatographic elution appeared with two prominent peaks. Preliminary polyacrylamide gel electrophoresis revealed impurities of the peak fraction. The enzyme was purified further using DEAE- cellulose II with a linear gradient of NaCl (0.5M). The active fractions were pooled. Enzyme activity was eluted from this column with a single peak symmetrically corresponding to the major protein peak (Figure 1) with the contaminant proteins being removed resulting in the final purification fold of 20.58 and a recovery of 4.7%. This preparation was used for the further application studies. The similar pattern of studies were earlier performed in *S. sobrinus* where 27 fold of purification was reported [14].

Determination of molecular weight

Molecular weight of the proteins were determined by electrophorizing the purified dextranase enzyme on SDS- polyacrylamide gel. In the present study *S. sobrinus* yielded a molecular weight of 50 kDa **(**Figure 2) which is higher when compared to the previous report of *S. sobrinus* yielded a molecular weight of 43 kDa [16]. The separation of one major protein band on analytical SDS- PAGE corresponds to a single band of activity. The estimation of molecular weight for each dextranase fraction indicates the separation of dextranase complex into the monomeric-molecular weight forms of the enzyme [13]. Dextranase protein is one of a number of proteins that exhibit aberrant electrophoresis migration in SDS- gels. About half (2.2 kb) of the dex gene appears to be needed to specify enzyme activity [17, 18, 19].

Table 1
Purification of dextranase enzyme from S. sobrinus

Fractions	Total Activity (U)	TotalProtein (mg)	Specific Activity (U)	Purification (Fold)	Recovery (%)
Culture filtrate	44000	4360	10.18	1	100
Ammonium sulphate precipitation	20915	667.0	31.35	3.07	47.10
Dialysis	12930.4	210.7	61.40	6.03	29.12
DEAE-cellulose column chromatography I	6922.5	54.0	128.19	12.59	15.59
DEAE- cellulose column chromatography II	2096	10.0	209.60	20.58	4.70

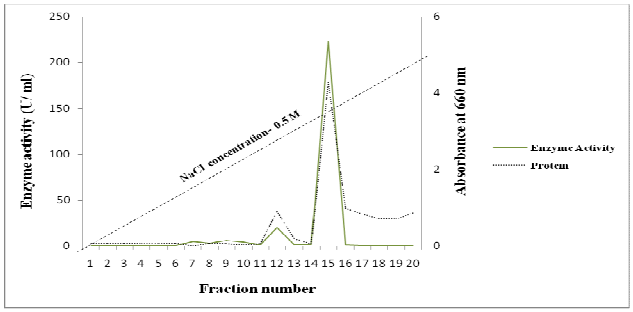

Figure 1
Chromatography of partially purified fractions of dextranase enzyme by S. sobrinus on DEAE- cellulose column II

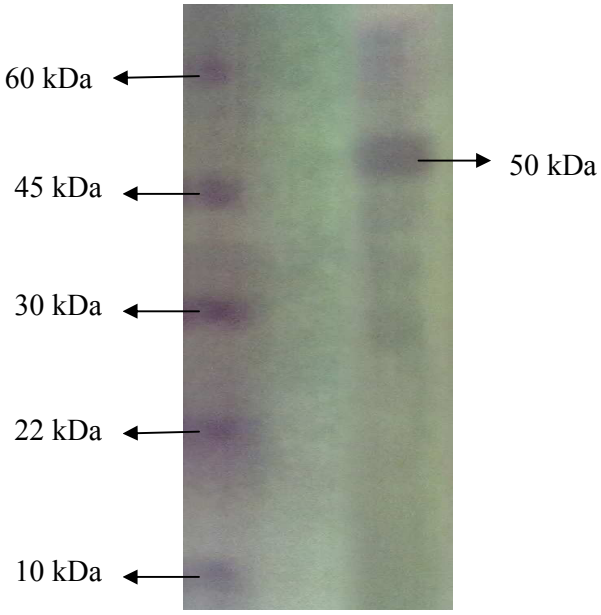

Figure 2
SDS- polyacrylamide gel profile of dextranase

CONCLUSION

In the present study dextranase enzyme production from *S. sobrinus* isolated from the human dental plaque was exploited by submerged fermentation in tryptic soy broth. The enzyme was purified with the purpose to obtain an alternative to available dextranase in the toothpaste industry.

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REFERENCES

1. Maripandi A, Arun Kumar T and Al Salamah AA, Prevalence of dental caries bacterial pathogens and evaluation of inhibitory concentration effect on different tooth pastes against *Streptococcus* sp, Afr. J. Microbiol. Res., 5 (14), 2011, 1778-1783.

2. Beighton D, The complex oral microflora of high- risk individuals and groups and its role in the caries process, Comm. Dent. Oral Epidemiol., 33, 2005, 248-255.
3. Nascimento MM, Lemos JAC, Abranches J, Goncalves RB and Burne RA, Adaptive acid tolerance response of *Streptococcus sobrinus*, J. Bacteriol., 189 (19), 2004, 6383-6390.
4. Fitzgerald RJ and Jordan HV, Polysaccharide producing bacteria and caries, In Harris RH (ed.), Art and science of dental caries research, Academic Press Inc.,New York 1968, 79-87.
5. Gibbons RJ, Formation and significance of bacterial polysaccharides in caries etiology, Caries Res.,2,1968,164-171.
6. Khalikova E, Susi P and Korpela T, Microbial dextran -hydrolyzing enzymes: Fundamentals and applications, Microbiol. Mol. Biol. Rev., 69 (2), 2005, 306-325.
7. Singleton V, Horn J, Bucke C and Adlard M, A new polarimetric method for the analysis of dextran and sucrose, J. Am. Soc. Sugarcane Technol., 22, 2002, 112-119.
8. Horita M, Katsuro T, Obayashi K and Akahori H, Dextranase production in a "fed- batch" culture, (Studies on dextranase production, Part 1), Nippon Nougeikagaku Kaiai, 63, 1989, 837-844.
9. Hirasawa M, Takada K, Method of isolating *Streptococcus sobrinus* and selection medium, US Patent 7,169,579 B2, 2007.
10. Staat RH and Schachtele CF, Evaluation of dextranase production by the cariogenic bacterium *Streptococcus mutans,* Infect. Immun., 9 (2), 1974, 467-469.
11. Janson JC and Porath J, Enzymatic assay of dextranase (EC 3.2.1.11), Methods Enzymol., 8, 1966, 615.
12. Lowry OH, Rosebrough NJ, Farr AC and Randall RJ, Protein measurement with Folinphenol reagent, J. Biol. Chem., 1951, 265-275.
13. Barrett JF, Barrett TA and Curtiss R III, Purification and partial characterization of the multicomponent dextranase complex of *Streptococus sobrinus* and cloning of the dextranase gene, Infect. Immun., 55 (3), 1987, 792-802.
14. Yamashita Y, Hanada N and Takehara T,Purification of a fourth glucosyltransferase from *Streptococcus sobrinus,* J. Bacteriol., 171 (11), 1989, 6265-6270.
15. Laemmli UK,Cleavage of structural protein during the assembly of headof bacteriophage T4, Nature, 227, 1970, 680-685.
16. Sun JW, Wanda SY and Curtiss R III, Purification, characterization and specificity of dextranase inhibitor (Dei) expressed from *Streptococcus sobrinus* UAB108 gene cloned in *Escherichia coli*, J. Bacteriol., 177 (7), 1995, 1703-1711.
17. Barrett JF, Barrett TA and Curtiss R III, Biochemistry and genetics of dextranase from *Streptococcus mutans* (sic *sobrinus*) 6715. In Hamada S, Michalek SM, Kiyono H, Menaker L, and McGhee JR (eds.), Molecular microbiology and immunology of *Streptococcus mutans.* Elsevier Science Publishing, Inc., New York, 1986, 205-215.
18. Curtiss R III, Goldschmidt R, Barrett J, Thoren-Gordon M, Salzberg DJ, Murchison HH and Michalek S,Genetic analysis of surface proteins essential for virulence of *Streptococcus sobrinus*. In Ferretti JJ and Curtiss R III (eds.), Streptococcal genetics. Am. Soc. Microbiol., Washington D.C., 1987, 212-216.
19. Wanda SY and Curtiss R III, Purification and characterization of *Streptococcus sobrinus* dextranase produced in recombinant *Escherichia coli* and sequence analysis of the dextranase gene, J. Bacteriol., 176 (13), 1994, 3839-3850.