Isolation and Purification of glucosyltransferase from mutans *Streptococcus Sob*rinus(serotype G) local isolate.

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Abstract

Background: Glucosyltransferase is an extracellular enzyme produced by mutans streptococci responsible for polymerizing the glucose moiety of sucrose to form glucan**.**

Objective: Isolation and purification of glucosyltransferase from mutans *Streptococcus sobrinus*.

Methods: The enzyme was purified from mutans *Streptococcus sobrinus* by ultrafiltration , adsorption chromatography, ion-exchange by DEAE-cellulose and gel filtration by Sephacryl S-200.

Results: Large scale production, concentration and purification of mutans streptococci (*S.sobrinus*) (serotype G) N_{10} glucosyltransferase (GTF) were done by ultrafiltration-method using an Amicone-filter P50,adsorption hromatography (hydroxyapatite beads), ion-exchange chromatography (DEAE-cellulose column) and gel-filtration chromatography using (Sephacryl S-200) column. Three purified GTF enzymes (GTF-I_a, GTF-I_b, GTF-II) were detected with a specific activity of (31.60; 31.50 and 66.270) Unit/mg protein respectively and the fold of purification are (27.59; 27.92 and 58.75 respectively with yield of enzymes (14; 10.94 and 17.11 %) respectively.

Conclusion:The purified enzyme with accepted yield may open new approaches for its using in oral passive immunization against dental caries in experimental animals by using hen egg yolk antibodies specific for cell associated GTF of mutans streptococci bacteria.

Keywords: glucosyltransferase , *Streptococcus sobrinus*, purification, adsorption chromatography.

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Introduction

Glucosyltransferase is an extracellular enzyme produced by mutans streptococci responsible for polymerizing the glucose moiety of sucrose to form glucan which plays an important role in caries formation process [1, 2]. Several methods have been used for purification of mutans streptococci GTF enzyme. Challacombe [3] purified glucosyltransferase (GTF) enzyme from culture fluid of *S. mutans* by the use of hydroxyapatite column chromatography. Stepwise elution in 0.2 M and 0.5 M phosphate buffer resulted in two pools of activity as determined by isoelectrical focusing of this preparation revealed it to be a mixture of at least seven GTFs.

Other purification procedures were made from 20L culture supernatant of *S. mutans* by filtration through different ultrafiltration membranes in an Amicon Ultrafiltration cells in order to concentrate the enzyme and to remove any contaminating D-glucan. Polyacrylamide gel electrophoresis was used in order to quantitate the enzyme acitivity and the degree of purification [4-6].

Taubman [7] purified two types of GTF enzymes from *S. sobrinus* using SDS-PAGE and named them GTF-I and GTF-S with molecular weights of 153 KDa and 148 KDa, respectively. Purification procedures were

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performed by Sephadex G-100 column, Sepharose 4B-CL, and then the two enzymes were separated on a column of DEAE-Bio gel A as two peaks.

Purification of *S. sobrinus* GTF enzymes was also done from culture supernatant of this bacterium by chromatography on Sephadex G-100. The GTF-rich pools were then subjected to fast protein liquid chromatography on Superose 6. The gel filtration step separates non-GTF and other glucan-binding proteins as demonstrated by (SDS-PAGE). *S. sobrinus* GTF preparation obtained after gel filtration on Superose 6 contained a mixture of water (GTF-I)-insoluble glucan product [IG], (GTF-U) primer stimulated soluble glucan [SG] product and (GTF-S) primer independent SG-product [8, 9].

Methods

Bacterial local isolate *S. sobrinus* (serotype G) N10 which was isolated and identified as GTF producers isolate by Al-Mudallal [10] was grown on the surface of blood agar medium and incubated anaerobically at 37°C for 48 hrs.

A (2.5 ml) of this stock culture was inoculated into (250 ml) Todd-Hewitt broth medium containing (1.8%) glucose and incubated anaerobically for 18-24 hrs at 37°C [11].

Determination of GTF activity

The amount of glucan produced by GTF was estimated following the method of phenol-sulfuric acid by Debois [13].

The protein concentration was estimated in the supernatant by Bradford [12].

Extraction of GTF

Large scale production of GTF was done from the chosen bacterial isolate after growing in (750ml) Todd-Hewitt broth medium. Total viable count was determined for the stock bacterial culture (O.D. =0.25) by making a serial dilutions of the bacterial growth (10^{-1}-10^{-6}). After the extraction of GTF from bacterial culture was done the GTF activity and protein concentration as well as specific activity were calculated in (10 ml) of bacterial suspension.

The producible crude GTF was passed through an Amicon-filter P50 in (Ultrafiltration-cell) and concentrated to (40 ml). GTF activity and protein concentration then specific activity were also determined in (10 ml) of the concentrated suspension.

Purification of GTF by Adsroption Chromatography

The purification of GTF was done by using adsorption chromatography using hydroxyapatite beads which was prepared and packed with the enzyme according the manufacturing company (Bio-Rad-USA) and following the batch-wise method described by Scopes [14]. The 40 ml of crude concentrated GTF enzyme were added at (5°C) with a gentle stirring and left for a time to adsorb the enzyme with the beads. This mixture was then transferred to an Amicon-filter P50 in (Ultrafiltration-cell) and filtrated under pressure. To the remaining precipitates on the filter washing was made by the addition of 25 ml of 0.15M phosphate buffer pH 7.5 and fractions of 5 ml were collected from the out-part of the filter. Then 25 ml of 0.3M phosphate buffer pH 7.5 were used to elute the protein from the remaining precipitate on the filter then fractions of 5 ml were collected.

The presence of GTF was estimated by measuring GTF activity for all fractions which represented the washing and elution parts then after collection of active GTF fractions together, protein concentration, GTF activity and specific activity were determined.

The exchanger DEAE-cellulose was prepared and packed into a

column (7.5 x 3.5 cm) following the method described by Whitaker [15].

Partially purified concentrated GTFs 12 ml were separately passed after loaded onto the column carefully. Then 100 ml of 0.05M phosphate buffer pH 7.5 were added. Proteins were eluted by using 200 ml of a gradient from 0.05-0.3 M phosphate buffer pH 7.5. Fractions of 5 ml were collected and absorbency was monitored at 280 nm. The presences of the GTFs was estimated from each fraction of the major peaks then protein concentration and specific activities were determined.

To Sephacryl S-200 column (67x2.1cm), a 3 ml sample of each concentrated partially purified GTFs were added to the column, Elutions of proteins were done with the application of 200 ml of 0.3 M phosphate buffer pH 7.5. A 5 ml fraction was collected and the absorbency was monitored at 280 nm.

Different standard proteins (Thyroglobulin, ferritin , catalase, aldolase, bovine serum albumin with molecular weight 660000,440000, 230000, 158000 and 67000 respectively were applied through Sephacryl S-200.

Results

Before the purification process, large scale production of GTFs was done from mutans streptococci (*S.sobrinus*) N_{10} bacteria chosen isolate. After extraction of GTF, protein concentration, GTFs activity then specific activity were determined in 10 ml of bacterial suspension. Results showed that bacteria about $1x10^8$ cells/ml were able to produce 0.7 mg/ml of crude GTFs with an activity of 0.790 U/ml which had a specific activity of 1.128 U/mg protein after 1 fold of purification, when 750ml crude GTFs concentrated by an Amicon-filter P50 in Ultrafiltration-cell to 40ml . Table 1 indicates that protein concentration and GTFs activity were recorded to be 0.601 mg/ml and 11.076 U/ml with a specific activity of 18.42 U/mg protein after 16.32 folds of purification which represented 74.77% yield of enzyme.

Purification of GTFs was done by Adsroption chromatography using hydroxyapatite beads. Results showed that when washing with 25 ml of 0.15 M phosphate buffer pH 7.5 then elution with 0.3M of the same buffer, GTFs activity appeared in all fractions of the washing and elution parts. The collection of fractions of the washing part as well as for fractions of the elution part in separated sterile containers was done. GTFs activity, protein concentration and specific activity which were determined for these separated parts. Table 1 indicates that washing with 0.15M phosphate buffer produced a GTF-I activity of 6.699 U/ml, with a specific activity of 18.66 U/mg protein after 16.54 folds of purification which represented 28.265% yield of enzyme. The elution with 0.3M phosphate buffer produced a GTF-II activity of 6.922 U/ml, protein concentration of 0.365 mg/ml and a specific activity of 18.96 U/mg proteins after 16.80 folds of purification which represented 29.206 % yield of enzyme.

Accordingly,adsorption chromatography (hydroxyapatite beads) is capable to produce two GTF enzymes with very close activity and protein concentration values. These two enzymes were named GTF-I and GTF-II. GTF-I represented the collection of fractions after washing and GTF-II represented the collection of fractions after elution. Purification of GTF enzymes (GTF-I and GTF-II) were done by ion-exchange chromatography (DEAE-cellulose column). 12ml of the concentrated samples from the previous step (GTF-I and GTF-II) were passed separately through the

DEAE cellulose column. Results shown in figures (1) and (2) indicate that washing with 100 ml of 0.05M phosphate buffer pH 7.5 allowed the presence of two peaks which were represented by fractions 9-16 for GTF-I and fractions 11-20 for GTF-II. Then after elution of proteins with 200 ml of a gradient from 0.05M to 0.3M phosphate buffer pH 7.5, two peaks were obtained for GTF-I which were represented by fractions 20-26 and 32-38 and one peak was obtained for GTF-II represented by fractions 47-52. Each fraction of GTF-I and GTF-II which represented the peaks after washing and elution processes were tested for GTF activity. Accordingly, only fractions 20-26 and 32-38 of GTF-I and fractions 47-52 of GTF-II were able to reflect GTF activity.

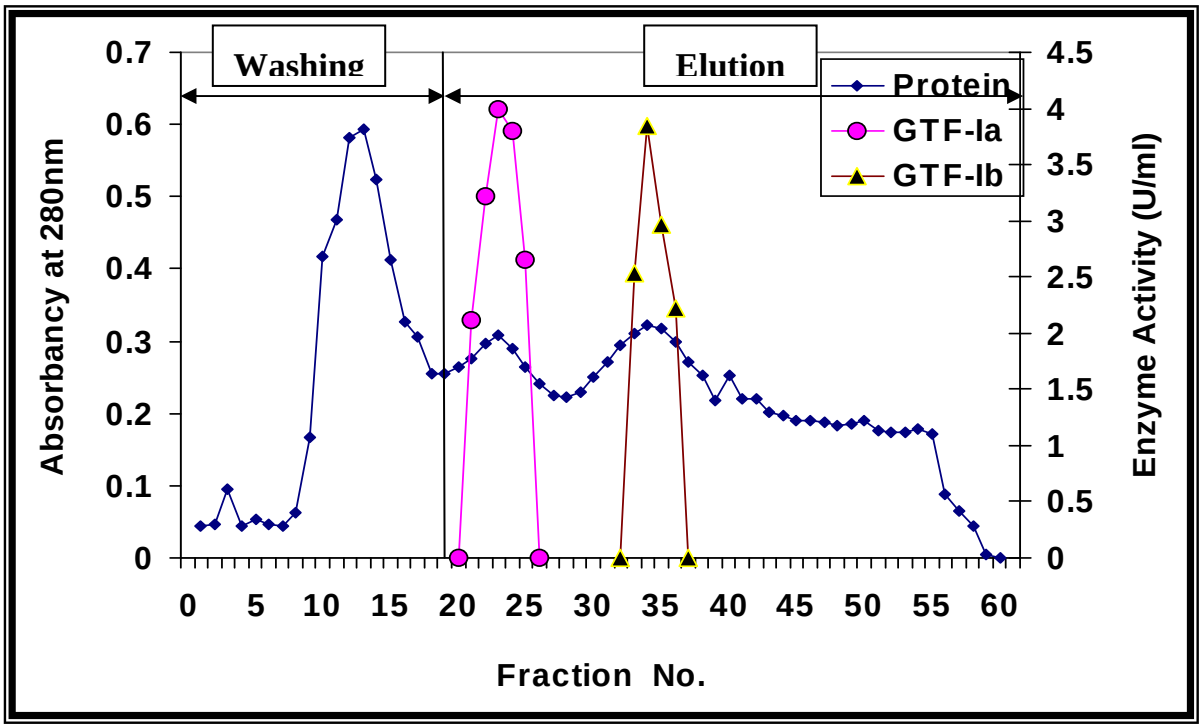

Figure 1: Purification of GTF-I enzyme by ion exchange chromatography (DEAE-Cellulose) column (7.5x3.5cm). The column was washed by using (0.05M) phosphate buffer pH (7.5), and then eluted by using a gradient of (0.05M) to (0.3M) phosphate buffer pH (7.5).

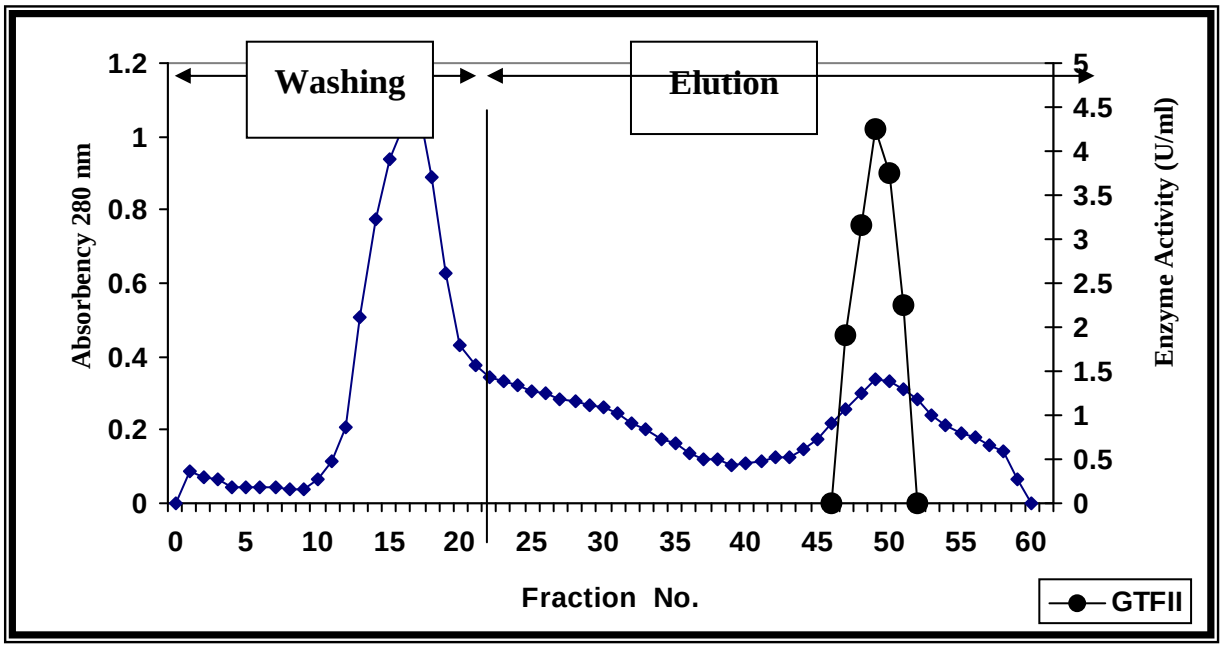

Figure 2: Purification of GTFII enzyme by ion exchange chromatography (DEAE-Cellulose) column (7.5x3.5cm).The column was washed by using (0.05M) phosphate buffer pH (7.5), and then eluted by using a gradient of (0.05M) to (0.3M) phosphate buffer pH (7.5).

Table (1), indicates that specific activities of (17.52 U/mg protein), 22.464 U/mg protein with purification folds of 15.53, 19.91 and yield of 15.088%, 13.269% were obtained respectively for GTF-I_a and GTF-I_b. For GTF-II a specific activity of (26.88 U/mg protein) was obtained with purification folds of 23.82 and yield of GTF of 17.353%.

Accordingly, three GTF enzymes (GTF-I_a, GTF-I_b and GTF-II) were obtained after purification with ion-exchange chromatography. Partially purified (GTF-I_a, GTF-I_b and GTF-II) were passed separately through an Amicon-Filter P50 in (Ultrafiltration-Cell) to concentrate them to (5 ml).

Figures 3, 4 and 5 indicate the presence of six peaks (two for GTF-I_a, three for GTF-I_b and one for GTF-II) . After the determination of GTF activity for all these peaks it is clear that fractions (30-33) of GTF-I_a, Fractions (32-36) of GTF-I_b and fractions (25-28) of GTF-II were able to produce GTF enzyme. Fractions (30-33) of GTF-I_a, fractions (32-36) of GTF-I_b and fractions (25-28) of GTF-II were pooled separately for each enzyme then GTF activity, protein concentration and specific activity were determined.

Results shown in table (1), indicate that GTF-I_a, GTF-I_b and GTF-II were able to reflect GTF activity, and specific activity of (5.531 U/ml), (4.320 U/ml), (6.760 U/ml);; (31.60 U/mg protein), (31.50 U/mg protein), (66.270 U/mg protein) after (27.59), (27.92), (58.75) folds of purification and yield of 14.001%, 10.936% and 17.113% respectively.

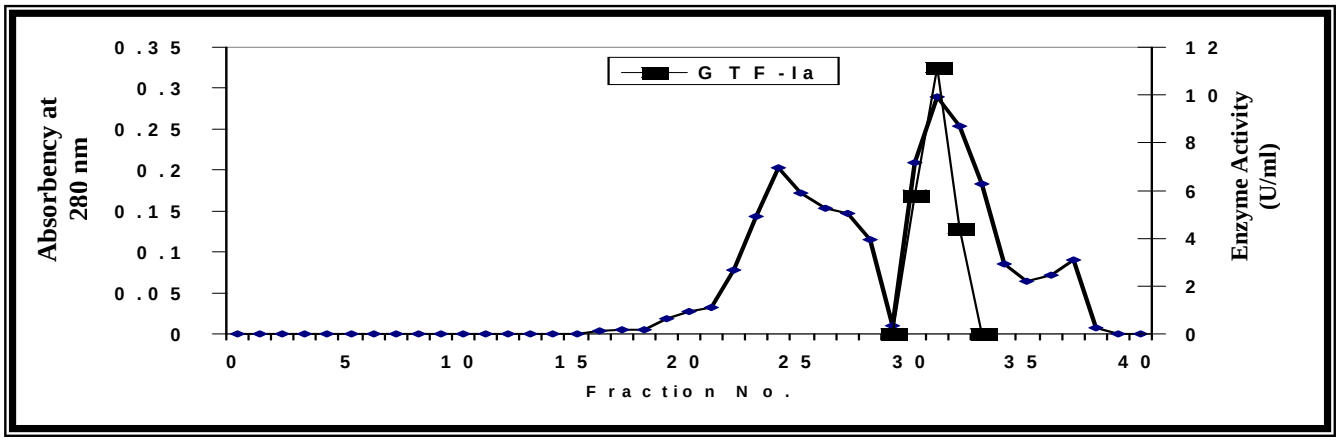

Figure 3: Purification of GTF-I_a by gel-filtration chromatography (Sephacryl S-200) column (67x2.1cm). Eluent: (0.3M) phosphate buffer pH (7.5) at a flow rate of (50ml/hour).

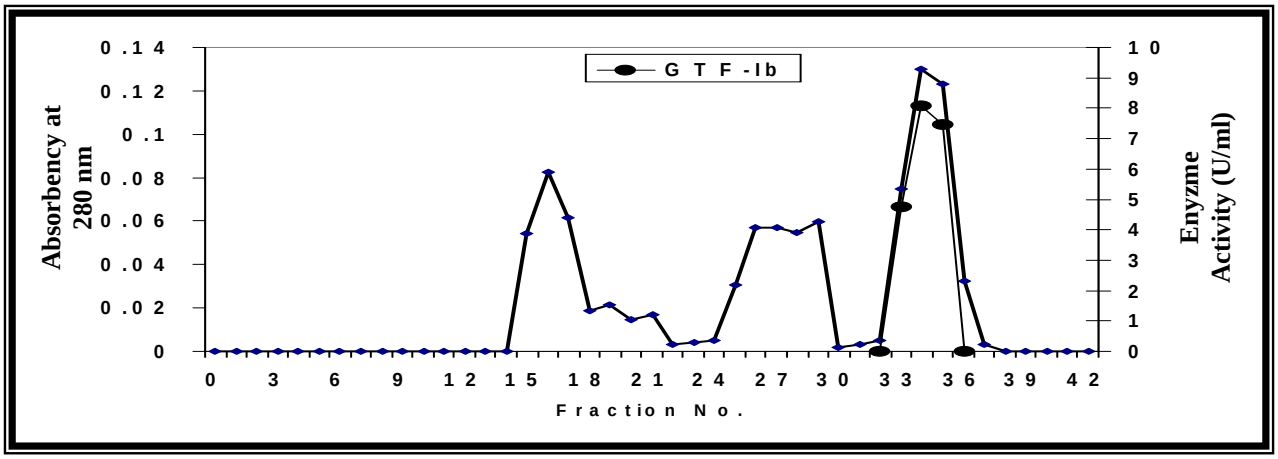

Figure 4: Gel filtration column chromatography (Sephacryl S-200) of GTF-I_b (67x2.1cm). Eluent: (0.3M) phosphate buffer pH (7.5) at a flow rate of (50ml/hour).

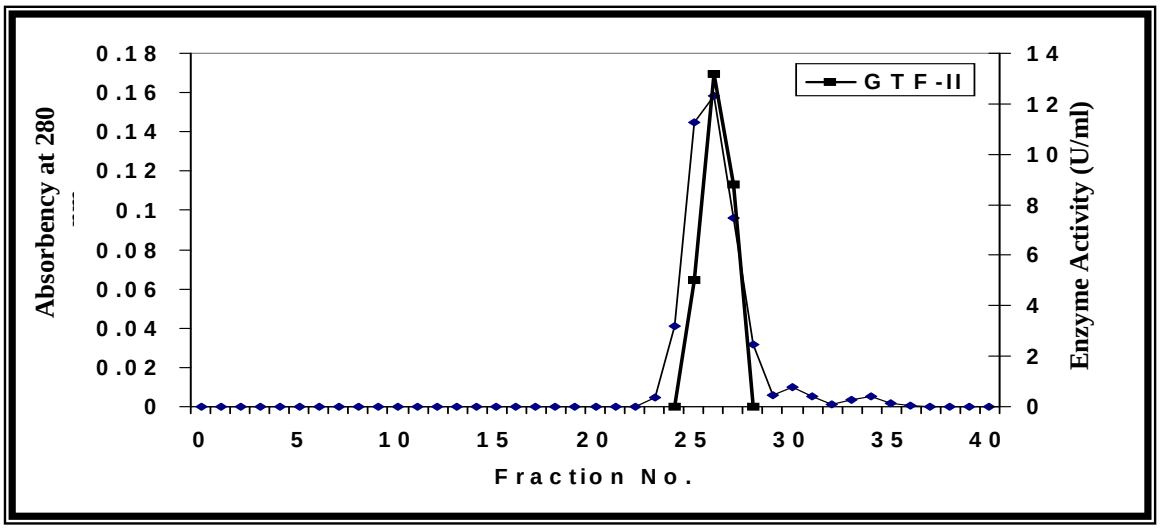

Figure 5: Purification of GTF-II enzyme by gel-filtration chromatography (Sephacryl S-200) column (67x2.1cm).

Table 1: Purification and yield of GTF enzymes from mutans streptococci (N_{10}) (*S. sobrinus*) (serotype G).

Steps	Volume (ml)	Enzyme activity (U/ml)	Total activity (U)	Protein Concen. mg/ml	Specific activity (U /mg)	Purification factor (fold)	Yield (%)
Crude Enzyme	750	0.790	592.50	0.7	1.128	1	100
Concentrated crude GTF by Amicon Filter	40	11.076	443.05	0.601	18.42	16.32	74.77
Adsorption chromatography hydroxyapatite beads (Batch wise)							
GTF-I	25	6.699	167.475	0.359	18.66	16.54	28.265
GTF-II	25	6.922	173.05	0.365	18.96	16.80	29.206
DEAE-Cellulose column chromatography							
GTF-I_a	25	3.576	89.40	0.204	17.52	15.53	15.088
GTF-I_b	20	3.931	78.62	0.175	22.46	19.91	13.269
GTF-II	25	4.113	102.82	0.153	26.88	23.82	17.353
Gel-filtration Sepharcryl S-200 column chromatography							
GTF-I_a	15	5.531	82.96	0.175	31.60	27.59	14.001
GTF-I_b	15	4.320	64.80	0.137	31.50	27.92	10.936
GTF-II	15	6.760	101.40	0.102	66.27	58.75	17.113

Discussion

Figures and Edwards [16] concentrated their GTF from three liters bacetrial culture (*S. mutans*) by using an Amicon "on-line" column effluent-concentration (Amicon-module (ECI) equipped with a PMI10 Ultrafiltration membrane). After concentration to (60 ml), protein concentration, GTF activity and specific activity were (0.660 mg/ml), (4 U/ml) and (12 U/mg protein) respectively.

Al-Hayali [17] began the purification procedure of GTF of mutans streptococci (BiotypeI- *S. mutans*) and mutans streptococci (Biotype IV-*S. sobrinus*) with precipitation by saturated ammonium sulfate from (450 ml) and (400 ml) respectively. After precipitation, GTF activity and specific activity values for each were recorded to be 0.315 U/ml; 0.406 U/ml; 0.16 U/mg proteins and 0.15 U/mg protein respectively. Three types of GTF were obtained after the purification step by gel-filtration chromatography using (Sepharose CL-6B). The purification scheme of this step for the third GTF reflected GTF activity, protein concentration and specific activity of 0.208 U/ml, 0.09 mg/ml and 2.3 U/mg protein after 153.3 folds of purification with yield of 20.8% respectively.

Koga [18] purified GTF of mutans streptococci (*S. sobrinus*) (serotype D) by hydroxyapatite column after precipitation with 50% saturated ammonium sulfate. GTFase-S and GTase-I were separated with specific

activities of 3.7 U/mg protein and 1.8 U/mg protein respectively.

Yamashita [19] described the purification of four glucosyltransferase from mutans streptococci (*S. sobrinus*) (serotype G) by DEAE-cellulose chromatography. GTF fractions were collected from the first DEAE-cellulose each separately and entered to the second DEAE-cellulose column. The specific activity, fold of purification and yield (%) for [P_2] (one kind of glucosyltransferase enzymes) after the first and second DEAE-cellulose were recorded to be 2.39 U/mg protein, 8.35 U/mg protein; 8.54 and 29.8 folds of purification with 43.6% , 27.0% yields of GTF respectively.

Turchi and Edwards [20] characterized and purified GTF from *S. mutans* (serotype C). The last step in the purification procedure was Gel-filtration chromatography with the use of Bio-Gel A1.5cm. The purification scheme described the presence of three GTF enzyme with specific activity were recorded to be 37 U/mg protein, 208 U/mg protein, 178 U/mg protein after 25 , 140 and 120 folds of purification with of yield of 50%.

According to the specific activities, fold of purification and yields of enzyme purification of GTF by gel filtration chromatography using (Sephacryl- S-200) column is more efficient than purification by (Sepharose CL-6B) and less efficient than purification by (Bio-Gel A1.5 cm).

References

1. Tsumori M and Kuramitsu H. The role of *Streptococcus mutans* glucosyltransferase in the sucrose dependent attachment to smooth surfaces: essential role of the GTF-C enzyme. Oral microb. Immun. (1997) 12: 274.

2. Wunder D E and Bowen W H. Action of agents on glucosyltransferase from *Streptococcus mutans* in solution and adsorbed to experimental pellicle. Arch. Oral Biol. 1999; 44: 203-214.

3. Challacombe S J, Guggenheim B and Lehner T. Antibodies to an extract of Streptococcus mutans containing glucosyltransferase activity, related to dental caries in man. Arch. Oral Biol. 1973; 18:657-668

4. Ciardi J E, Beaman A J and Wiltenberger C L. Purification, resolution and interaction of the glucosyltransferase of *Streptococcus mutans* 6715. Infect. Immun. 1977; 18(1): 237-246.

5. Figures W R and Edwards J R. Improved purification procedure for the extracellular D-Glucosyltransferase from *Streptococcus mutans*. 6715. Carbohy. (1978). Res.30:248-251.

6. Smith D J, Taubman M A and Ebersole J L. Preparation of glucosyltransferase from *Streptococcus mutans* by elution from water-insoluble polysaccharide with a dissociating solvent. Infect. Immun. (1979). 23: 446-452.

7. Taubman M A, Smith D J, King W F, Eastcott J W, Bergey E J. and Levine M J. Immune properties of glucosyltransferases from *S. sobrinus*. J. Oral. Path. 1988; 17: 466-470.

8. Taubman M A, Smith D J, Holmberg C J and Eastcott J W. Coimmunization with complementary glucosyltransferase peptides results in enhanced immunogenicity and protection against dental caries. Infect. Immun. 2000; 68: 2698-2703.

9. Smith D J, Barnes L A, King W F, Peacock Z, Wise D L, Trantolo D J. et al. Remote glucosyltransferase microparticle vaccine delivery induces protective immunity in the oral cavity. Oral Microbiol. and Immunol. 2003; 18: 240-248.

10. Al-Mudallal N H. Characterization of Glucosyltransferase of Mutans *Streptococci Streptococcus sobrinus* (Serotype G) functional and immunological assessment. PhD. Thesis. College of Scince, Al-Nahrain University (2006).

11. Christine D, Yuan-Wu Tai, S and Slade H D. Properties of *Streptococcus mutans* grown in a synthetic medium. Binding of glucosyltransferase and *in vitro* adherence, and binding of dextran / glucan, glycoprotein and agglutination. 1979; 23(3): 600-608.

12. Bradford M M. A rapid and sensitive method for the quantitation of microorganism's protein utilizing the principle of protein-dye binding. Analytical Bioch. 1976; 72: 248-254.

13. Dubois M, Gilles K A, Hamilton J K, Rebers P A and Smith F. Colorimetric method for determination of sugars and related substances. Anal. Chem. 1956; 28: 350-356.

14. Scopes R K. Inorganic adsorbents. In: Protein Purification Principals and Practice. 2nd

ed. SpringoVerlag. (1972). New York. PP. 675-678.

15. Whitaker J R. Principles of enzymology for the Food Science. Mocel Dekker, Inc. New York (1972).

16. Figures W R and Edwards J R. D-glucosyltransferase of *Streptococcus mutans*, Isolation of two forms of the enzyme that bind to insoluble dextran.Carbohydrate. (1981). Res. 88: 107-117.

17. Al-Hayali A M. Isolation and purification of gluosyltransferase from mutans streptococci and the relation to dental caries, dental plaque and parameters of saliva. Ph.D. (2002).Thesis, College of Dentistry, University of Baghdad.

18. Koga T, Sato S, Tnoue M, Takeuchi K, Furuta T and Hamada S. Role of primers in glucan synthesis by gluosyltransferases from *Streptococcus mutans* strain OMZ 176. J. Gene. Micorbio. (1982). 129: 751-754.

19. Yamashita Y, Hanada N and Takehara T. Purification of a forth glucosyltransferase from *Streptococcus sobrinus*. J. of Bact. (1989). 171(11): 6265-6270.

20. Turchi S L and Edwards J R. Characteristics of glucosyltransferase from cultures of *Streptococcus mutans* 6715 grown in Trypticase soy broth and chemically defined media. Curr. Microbiol. (1985); 12: 135-140.