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Original Research Article

Isolation and Identification of *Streptococcus mutans* (H5) produced glucosyltransferase and cell-associated glucosyltransferase isolated from dental caries

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A B S T R A C T

Streptococcus mutans have been proposed as the main etiological agents of dental caries and high levels of mutans streptococci in the plaque is correlated with a higher risk for dental caries. Seveenty five plaque samples are collected from human teeth. Forty two samples are considered to be positive bacterial isolates using MS-agar (Mitist Salivares agar). Thirty five isolates are considered belonging to the group Streptococci, among this isolates twenty nine isolates are expected to belonging to mutans streptococci group according to ability of producing special kind of exopolysaccharides. Twelve isolates consider as *S.mutans* with percentage (41%) depending to the staining with triphenyltetrazolium chloride and tolerance with NaCl, six isolates classified as serotype C by using Lancefield grouping identification. These isolates are tested for production of extracellular Glucosyltransferase (GTF) and cell-associated Glucosyltransferase (CA-GTF) through determination of their enzyme specific activity. All isolates are able to produce the enzyme; Streptococci isolate (H5) which identified as (*Streptococcus mutans* serotype C) is selected as the best producible isolate for GTF with a specific activity of (2.6 U/mg). It finds that GTF of the chosen isolate (H5) is produced during the middle stationary phase (18-35 hr.) and its maximal productivity is reached at (22 hr.).

Keywords

Streptococcus mutans
Exo-polysaccharides
Triphenyl-tetrazolium chloride
Glucosyl-transferase (GTF) and cell-associated Glucosyl-transferase (CA-GTF)

Introduction

Most strains of MS mutans especially *S.mutans* but not *S. sobrinos* (Balakrishnan, *et al*., 2000) produce intracellar iodine-staning polysaccharides (IPS) from sucrose which typically resemble glycogen, IPS can be metabolized leading to continued acid production which, according to result from experiments with rats, may contribute to their virulence (Kuramitsu, 1993). Because of this intracellular polysaccharide storage, these cariogenic bacteria have the ability to continue fermentation in the absence of exogenous food supplies (Loeshe, 1986). It was shown that therapeutic agents that

diminish EPS and IPS concentrations in biofilms also reduce the development of dental caries in rats (Koo *et al.*, 2005), confirming the importance ofthese polysaccharides in *S. mutans* cariogenicity.

S. mutans synthesis exocellular polysaccharide (EPS) i.e. glucan, from the glucosylresiduse of sucrose by secretion glucosyltransferase (GTFs). It is well known that *S.mutans* has at least three GTFs (GTF-B, C, and D). (Nishimura, *et al*., 2012). GT-B and D mainly synthesize water-insoluble α-(1-3) - and water soluble α-(1-6)-glucan. GTF-C associates with insoluble and soluble glucan synthesis, which is controlled by the genes *gtfB, gtfC,* and *gtfD* (Koo *et al*., 2010; Monchois*etal*., 1999). The *gtfB* and *gtfC* genes are tandomly arranged on *S. mutans* chromosomal DNA. (Ueda and Kuramitsu, 1988). The nucleotide sequences of *gtf* genes from different oral streptococci comply with the same basic pattern. The GTFs are very large proteins of approximately 1300- 1700 amino acids long (Devulapalle *et al*., 1997). Streptococcal GTFs have two common functional domains. The amino-terminal portion, the catalytic domain, is responsible for the cleavage of sucrose, and the carboxyl-terminal portion, the glucan binding domain, is responsible for glucan binding (Colby and Russell, 1997). In addition the two genes share extensive nucleotide sequence homology.

The primary amino acid sequences of the streptococcal GTF enzymes are highly homologous. The synthesis of extracellular water-insoluble glucans from sucrose is necessary for the formation of dental plaque by *S. mutans* (Hamada and Slade, 1980). It is generally understood that this polymerization is catalyzed by two types of extracellular glucosyltransferase one synthesizing a water-soluble product from sucrose (GTF-S) and another synthesizing water-insoluble product from sucrose (GTF-I). These two types of GTF, when combined, synthesize a complex, highly branched, adherent, water-insoluble glucan (Walker, 1978).The GTF produced by *S. mutans* can be found either in the culture supernatant or on the cell surface in an associated form. Certain GTFs can bind to the cell surface of MS and promote cellular adherence via insoluble glucan synthesized *de novo* from sucrose (Horikoshi*et al*., 1995). The origin of cell-associated enzyme of sucrose-grown cells has not been determined, nor has the role of soluble enzyme in the formation of cell-associated enzyme been investigated (Horikoshi*et al*., 1995). Two form of cell-associated GTFs from the serotype c *S. mutans* strain GS-5 one enzyme was extracted by treatment 1 M-NaCl from cells grown in TH broth. The other GTFs was an intracellular enzyme released after distruption of cells (Kuramitsu, 2006).

Materials and Methods

Samples Collection

Twenty five plaque samples were collected from dentist college, Baghdad University, in sterile universal tubes containing 2ml normal saline and 1% yeast extract Samples were stored at least 12hrs. in a cool place before transported to the laboratory.

Isolation of Streptococci Bacteria

Point one milliliter of each sample collection were spread on MS-agar plates using sterile spreading glass. Cultures were incubated anaerobically, using anaerobic candle jar, for 48 hrs. at 37□C. Count of more than 250 colonies (10^4 cells/ml) was considered as positive samples (Friedrich, 1981).

Identification of Isolates

Colonies grown on MS-agar medium was spread on the blood agar plates and incubated anaerobically for two days. Subcultures were repeated several times in order to obtain pure cultures. The identification of *S.mutans* is based on (according to the information in Bergeys Manual of Determinative Bacteriology 9th ed.,1994) distinctive colonial morphology on selective and nonselective agar, Gram staining, distinctive cell shape on light microscopy, specific growth characteristics, and sugar fermentation. In addition to that *S.mutans* isolates was identified by the commercial biochemical test system API 20 strep, the identification also was depended on the dextran production test which was done according the method of Guthof(1970).

Dextran Production Test

A 2ml of TYS broth medium was inoculated with loopfull of bacterial culture then incubated anaerobically at 37°C for two days. The culture was centrifuged at 3000xg for 10 minutes, 0.1ml of supernatant of each culture was added to each three tubes and stirred with 0.3 ml of 10% sodium acetate. A 0.8-fold volume of acetone was added to tube 1; a 1.2 fold volume of ethanol to tube 2 and 1.5-fold volume of methanol was added to tube 3. Each tube was shacked for 3 minutes and observed. Flocculation in all three tubes or only in acetone and turbidity in the alcohols have indicated that formation the dextran.

Identification of Colonies

Identification of streptococcus mutans from other streptococci was done according to the procedure described by Gold (1975) as follows: The bacteria was plated on mitissalivaris agar and incubated anaerobically for 24 hrs at 37°C followed by overnight aerobic incubation at room temperature. The plate was then sprayed with a 10 % solution of mannitoland incubated aerobically for 3 hrs at 37°C. the plate was next sprayed with a 4% solution of 2,3,5 triphenyltetrazolium chloride and incubated aerobically for another hour at 37°C. A change in color to a dark pink has indicated that for the presence of *Streptococcus mutans* while colonies of other streptococci remain blue.

The Fermentation of Different Carbohydrates Sources

The bacterial isolates can fermented different carbohydrates sources were determined follow the method described by Fngold and Baron (1986).

Brain heart infusion broth supplemented with 10% of each (mannitol, sorbitol, sucrose and inulin). Brain heart infusion broth medium used as negative control, while Sucrose as positive control, all carbohydrate solutions were sterilized by filtration 0.45µm and added aseptically to the autoclaved brain heart infusion broth which contain a 0.02% of phenol red, the suspended media inoculated with the tested isolates and incubated anaerobically at 37°C for 72 hrs. The color of media has changed from red to yellow as compared with the controls indicated the ability of these bacteria to ferment these carbohydrates sources.

Tolerance to High Concentration of Sodium Chloride

The ability of bacterial isolates to growth in 4% NaCl was tested using TYS broth medium and incubated anaerobically at 37°C for 48 hrs. Turbidity is an indicator for

the ability of the bacterial isolates to tolerate this concentration of NaCl as compared with control which did not contain this concentration of NaCl.

Antibiotic Sensitivity Test

It was made by using disk diffusion method according to Baron *et al* (1994). Loopfull of bacterial isolate inoculated 10 ml of Brain heart infusion broth, the culture was incubated at 37°C for 24 hrs. A 0.1 ml of broth separated on the Muller Hinton agar plates. Using sterile glass spreader to streak the inoculum on the plate surface and placed at room temperature for 10 minutes to allow absorption of moisture. It was selected antibiotic disks of Bacitracin 30 µgram, Vancomycin 30 µgram, Optochin 50 µgram, Cefataxime were placed on the inoculated plates then incubated at 37°C for 24 hrs. After this period, the diameters of inhibition zones were recorded and measured in (mm) by a ruler. Results were compared with that described by the National Committee for laboratory Standards (NCCLs, 2001).

Identification of Serotype

It was used Pastorex^{TM} Strep kit for direct identification for C-Latex anti Streptococcus group C, from BIORAD Company.

Determination of GTF Activity

It was determined by estimation of the amount of glucan that was formed by the action of the enzyme; following used the phenol-sulfuric acid method (Dubois *et al.*, 1956). A 1 ml of GTF (crude or purified) was mixed with 1ml of 5% phenolwith shaking for 2-3minutes, then stopped the reaction by the addition of 5 ml concentrated sulfuric acid. The absorbance was measured at 490 nm. One unit (1U) of GTF was defined as the amount of enzyme catalyzing the incorporation of 1 µ mole of glucose from sucrose under the condition of experiment (Mukasa*et al*., 1982).

Determination of Protein Concentration

According to the method of Bradford (1976) and Stoscheck (1990), the protein concentration determined as follow: A 20µl of GTF crude was mixed with 50µl of 1 M NaOH with shaking for 2-3 minutes then 1 ml of Bradford solution was added with shaking. The absorbance was measured at 595 nm by spectrophotometer.

Results and Discussion

Seventy five plaque samples were collected from College of Dentistry, University of Baghdad. The bacterial isolate was cultured on selective media (Mitiis-Salivarius Agar), which has induced growth of streptococci group and inhibit other bacterial species. Forty two samples were considered to be positive; according to the Friedrich (1981) that bacterial count about 10^4 cells/ml. Isolates identification, on the genus level, has been depending on catalase test, microscopic examination and gram staining. The streptococci are spherical or ovoid occurring pairs or chains, stain gram-positive and catalase negative bacteria according to the Friedrich (1981).

Thusly thirty five isolates are expected to be belonging to streptococci. Advanced identification to determine species depending on colonies shape and the form on the surface of MitistSalivaris- agar. Colonies morphology resembling *S. mutans* in the MS agar were quantited and characterized according to the identification criteria described by Facklam (1977) and Coykendall (1989) *Streptococcus mutans* could be distinguished from other species by raised, convex, undulate, opaque, pal blue colonies, granular "frosted glass" appearance.

May exhibit a glistening bubble on the surface of colony due to excessive synthesis of glucan from sucrose (MacFadden,1985), while other colonies showed zooglleic form smooth, hard colonies embedded in agar that's maybe belong to the *S. sanguis* depending on Colman and Williams (1972) and (MacFadden, 1985).

Other type of colonies appeared large, mucoid colonies classical "gum-drop" this appearance due to formation of levan from sucrose these colonies expected belong to *S.salivaries*, the colonies that were small, flat, hard colonies with a dome center considered as *S. oralis* following the information by REMEL, (1990).Shawkat, (2010) found variation in colony morphology seems to be related to synthesizing cell surface materials, namely, the glucan capsule.

According to this information twenty nine isolates are expected of belonging to mutans streptococci group and six isolates were belonges to *S. sanguis*, *S.oralies*, *S. salivarius*. Further selective species accordance to their ability of producing special kind of exopoly saccharides as considered to detected species of mutans streptococci group was done. This polysaccharide had very important role in dental plaque matrix.

Results shown in table (1) proved that twenty nine isolates are capable of producing poly glucan, whereas the other six isolates are considered as levans producers, because no reaction was appeared with ethanol and methanol that indicate assign for the polyfructan-production according to Guthof (1970) and AL-Mudallal (2006). further specific identification down to distinguished *S.mutans* from other oral streptococci down by using the staining the cells with 2,3,5-triphenyltetrazolium chloride (TCC) and changed color to pink (appendix 1) due to hydrolysis of mannitol to the acid by mannitol-1-phosphate dehydrogenase (Brown and Wittenberger, 1973) thusly results proved that twenty four isolates are stained with pink color (positive result) and five isolates showed no change in color (negative result).

In addition to previous identification of the mutans streptococci species the tolerance of 4% NaCl was tested as considered criteria to distinguished species of the mutans streptococci from that associated with dental disease (Holt *et al*., 1994). It was presented as evidence that salt concentration are one of the essential physical factors influencing insoluble glucan formation and that high concentration of salt not only increased the velocity of glucan synthesis but also shift the glucan formed from water soluble to water insoluble, also stimulated glucosyltransferase activity up to 2.7 fold (Mukasa *et al*., 1982). Accordingly twenty one isolates with a tolerance 4% NaCl while eight isolates no growth.

The lipase hydrolyzed olive oil in the temperature range of 10-45 ºC, with a maximum at 30 ºC (Fig. 4b). The lipase was quite stable at temperature of 30 ºC and 37ºC, retaining 83.33% and 76% of the lipase activity after 1 h incubation. The enzyme also showed > 50% of the residual activity after 1 h incubation at 45ºC, whereas 100% activity was retained at 10ºC. Maia *et al*., (2001) also found that lipase from *F. solani* FS1 was stable below 35 ºC and above this significant losses were observed. Most *S. mutans* strain are also distinguished from other oral bacteria by specific nutritional capabilities, that is, the ability of most strains to utilize the polyols sorbitol and mannitol as the sole carbon sources (Janda and Kuramitsu, 1978).

Thus and accordance to the results in the previous table (3) sucrose could rapidly produced acids causing a rapid drop in pH, changing to the yellow color, followed by a gradual recovery toward the baseline plaque pH. Sucrose is considered the most cariogenic dietary carbohydrate because it is fermentable and also serves as a substrate for the synthesis of extracellular polysaccharides (EPS) and intracellular polysaccharides (IPS) in dental plaque (Bowen, 2002). It has been found that sucrose reduces the concentration of calcium, inorganic phosphorus and fluoride in the dental biofilm (PaseLeme*et al*., 2004). The production of glucan and large amount of lactic acid by fermentation of carbohydrate constitutes major virulence factor in the causation of dental caries. (Loesche, 1986).

It is found that four isolates dont able to ferment mannitol, sorbitol and inulin and because all MS group that are isolate from humans are capable to ferment mannitol, sorbitol and inuline except for *S.sobrinus* and *S.cricetus* in which 85% of straine are positive to sorbitol and inuline (Beighton*et al* 1991), therefore these isolates do not belong to mutans streptococci group and five isolates expected belong to *S.sobrinus* and *S. cricetus* bacteria because of their inability to ferment sorbitol and inuline.

Further testing has done for separating oral Streptococcus from that belong to *S,pneumonia* and pediococcus spp. by using antibiotics sensitivity test. Four kind of antibiotics were used in this study bacitracin, optochin, vancomycin and cefotaxime. The results appeare in table (3) that all isolates resistant to bacitracin except four while only two, five, isolates sensitive to optochin, vancomycin respectively. Also all isolates were sensitive to cefotaxime except two isolates (H7, H17). The bacitracin is very important to isolate mutans streptococci from other group, hence it is used with selective media, mitissalivaris bacitracin agar (MSB) as well as Whiley and Beighton(1998) recorded that bacitracin resistance was detected for all species of MS that were isolate from human with the exception of *S. cricetus* bacteria. Thus, two isolates are considered not belong to MS bacteria group because of their resistance to vancomycin and sensitivity to bacitracin and optochin antibiotic three isolates excpected belong to *S.cricetus* which related to MS because of their resistance to optochin and sensitivity to bacitracin and vancomycin. Forfurther specific classification in order to group *Streptococcus mutans* serotype by using Lancefield group to distinguished serotype C from other.

The result has showen that eleven Isolates among nineteen isolates belong to *Streptococcus mutans* and just six isolates classified as serotype C. Al- Mudallal (2006) found that 30 isolates are related to mutans streptococci among 45 plaque sample and Rathod et *al*., (2012) found that the isolation rate of *S. mutans* is significantly higher about (57%) among individuals with caries as compared the individuals without caries. According to the other epidemiology data in korea, *S. mutans* and *S. sobrinus*are detected in 93% and 33% of the dental plaque sample, respectively.

The prevalence of *S. mutans* and *S. sobrinus* is reported to be 72% and 61% in 77 japans aged 3-5 years old, respectively (Okada *et al*., 2002). Wu *et al*. (2003) also reported the prevalence of *S. mutans* and *S. sobrinus* in 126 Chinese dental plaques to be 75% and 57%, respectively.

The production of the enzyme from the streptococcus species of the isolates has done. From seventeenth isolates only twelve isolates is selected which depending on their productivity.

Table.1 Production of Exopolysaccharide Dextran and Levans by Bacterial Isolates

NO	Symbols	Ethanol	Methanol	Acetone
1	H_1	+	+	+
2	H_2	+	+	+
3	H_3	+	+	+
4	H_4	+	+	+
5	H_5	+	+	+
6	H_6	+	+	+
7	H_7	+	+	+
8	H_8	+	+	+
9	H_9	+	+	+
10	H_{10}	+	+	+
11	H_{11}	+	+	+
12	H_{12}	+	+	+
13	H_{13}	+	+	+
14	H_{14}	+	+	+
15	H_{15}	+	+	+
16	H_{16}	+	+	+
17	H_{17}	+	+	+
18	H_{18}	+	+	+
19	H_{19}	+	+	+
20	H_{20}	+	+	+
21	H_{21}	+	+	+
22	H_{22}	+	+	+
23	H_{23}	-	-	-
24	H_{24}	+	+	+
25	H_{25}	+	+	+
26	H_{26}	+	+	+
27	H_{27}	-	-	-
28	H_{28}	+	+	+
29	H_{29}	+	+	+
30	H_{30}	-	-	-
31	H_{31}	+	+	+
32	H_{32}	-	-	-
33	H_{33}	-	-	-
34	H_{34}	+	+	+
35	H_{35}	-	-	-

+ flocculent formation - No reaction

Table.2 The tolerance of the Streptococcal isolates to grow in the 4% NaCl

No	Symbols	Growth in 4% NaCl
1	H_1	+
2	H_2	+
3	H_3	+
4	H_4	-
5	H_5	+
6	H_6	+
7	H_7	+
8	H_8	+
9	H_9	+
10	H_{10}	+
11	H_{11}	+
12	H_{12}	-
13	H_{13}	-
14	H_{14}	-
15	H_{15}	+
16	H_{16}	+
17	H_{17}	+
18	H_{18}	-
19	H_{19}	+
20	H_{20}	-
21	H_{21}	+
22	H_{22}	+
23	H_{24}	+
24	H_{25}	-
25	H_{26}	-
26	H_{28}	+
27	H_{29}	+
28	H_{31}	+
29	H_{34}	+

+ Growth - No Growth

Table.3 The Fermentation of Sucrose, Mannitol, Sorbitol and Inuline by Streptococcal Isolates

No.	Symbols	Positive control	Mannitol	Sorbitol	Inuline
1	H_1	+	+	+	+
2	H_2	+	+	+	+
3	H_3	+	+	+	+
4	H_5	+	+	+	+
5	H_6	+	+	+	+
6	H_7	+	+	-	-
7	H_8	+	-	-	-
8	H_9	+	+	+	+
9	H_{10}	+	+	+	+
10	H_{11}	+	-	-	-
11	H_{15}	+	+	-	-
12	H_{16}	+	+	+	+
13	H_{17}	+	+	+	+
14	H_{19}	+	+	-	-
15	H_{21}	+	+	+	+
16	H_{22}	+	+	-	-
17	H_{23}	+	+	-	-
18	H_{24}	+	+	+	+
19	H_{27}	+	-	-	-
20	H_{28}	+	+	+	+
21	H_{29}	+	-	-	-

+ Change color from red to yellow - No Change

Table.4 Antibiotic Sensitivity Test of Streptococcal Isolates.

No	Symbols	Bacitracin	Optochin	Vancomycin	Cefotaxime
1	H_1	R	R	R	S
2	H_2	R	R	R	S
3	H_3	R	R	R	S
4	H_5	S	R	S	S
5	H_6	R	R	S	S
6	H_7	R	R	R	R
7	H_8	R	R	S	S
8	H_9	S	R	S	S
9	H_{10}	S	S	R	S
10	H_{11}	R	R	R	S
11	H_{15}	R	R	S	S
12	H_{16}	R	R	R	S
13	H_{17}	R	S	S	R
14	H_{19}	S	S	R	S
15	H_{21}	R	R	R	S
16	H_{22}	R	R	R	S
17	H_{23}	R	R	S	S
18	H_{24}	R	R	R	S
19	H_{27}	S	R	S	S
20	H_{28}	R	R	R	S
21	H_{29}	R	R	R	S

R: Resistance S: Sensitive

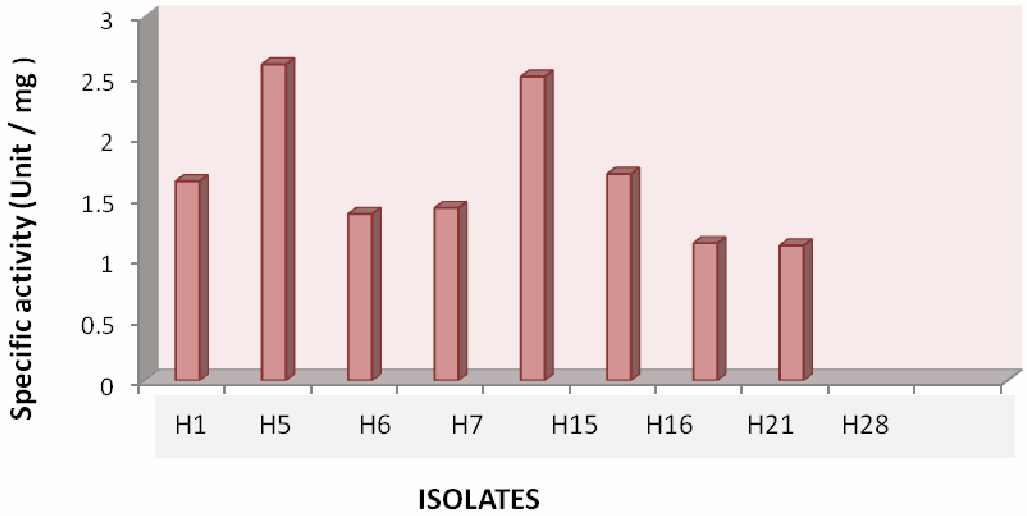
Table.5 Serotyping Group of Streptococcal Isolates

No	Symbols	Species	Serotype
1	H_1	*S. mutans*	Other
2	H_2	*S.mutans*	C
3	H_3	*S.mutans*	Other
4	**H_5**	*S. mutans*	C
5	**H_6**	*S. mutans*	C
6	H_7	Other	Other
7	H_9	Other	-
8	H_{10}	Other	-
9	H_{15}	Other	-
10	H_{16}	*S. mutans*	Other
11	**H_{17}**	*S. mutans*	C
12	H_{19}	Other	-
13	**H_{21}**	*S.mutans*	C
14	H_{22}	Other	-
15	H_{23}	Other	-
16	**H_{24}**	*S. mutans*	C
17	H_{28}	*S. mutans*	Other

Table.6 GTF Production from Different Mutants Streptococci Isolate

No	Symbols	Serotype	Activity (U/ml)	ProteinConc. (mg/ml)	Specific Activity (U/mg)
1	H_1	other	32.8	20	1.64
2	H_2	other	27.8	22.3	1.24
3	H_3	C	55.6	22.2	**2.5**
4	**H_5**	**C**	**65.7**	25	**2.6**
5	H_6	C	45.6	33.2	1.37
6	H_9	Other	42.6	44.8	0.95
7	H_{10}	Other	22.9	35.8	0.63
8	H_{16}	Other	32.9	19.3	1.7
9	H_{17}	C	19.4	23.5	0.82
10	H_{21}	C	36.2	31.9	1.13
11	H_{24}	C	26.9	33.9	0.79
12	H_{28}	Other	44.2	39.8	1.11

Figure.1 The Abilities of the Mutans Streptococci Bacterial Isolates to Express GTF.



It's appeared from the results shown in table (6) that all selected isolates are capable of producing an extracellular GTF enzyme and cell-associated GTF enzyme. Protein concentration and activity of GTF for isolates are made, and it's found that specific activity of GTF are ranged between (0.54 - 2.6 U/mg protein). According to the table (6) all isolates bacteria of mutans streptococci and serotype belonged this bacteria are capable to secret GTF, this result applied with numerous reports that confirmed mutans streptococci serotypes (A,B,C,D,F and G), *S. salivarias* and *S. sanguis* are capable to express several GTF types (Hamada *et al*., 1989). The specific activities of GTF of isolates (H_1, H_2, H_3, H_5, H_6, H_{16}, H_{21}, and H_{28}) are very approached to each other thus the conformation is made according to the choose the highest GTF producible bacteria among them.

The extraction of GTF from these isolates is repeated for them in 10 ml BHI broth medium and again determined protein concentration, specific activity. Result has shown in figure (1) indicate that all isolates are capable to produce an extracellular GTF enzyme and the specific activities of GTF for all isolates are ranged between (0.54-2.6 U/mg protein) and the isolate (H_5)serotype C has the highest GTF specific activity (2.6 U/ mg protein) followed by H_{15} (2.5 U/mg protein) then H_{16}, H_1, H_7, H_{21} with specific activity of (1.7, 1.6, 1.3, 1.24, 1.13 U/mg protein) respectively, wherefore it is chosen H_5 for large scale production and other characterization of GTF enzyme.

AL-Jumaily*et al* (2009) also indicated that all isolates (mutans streptococci) are capable to produce an extracellular GTF enzyme but the specific activities of GTF for all isolates are ranged between 0.083-0.51U/mg protein as well as they indicated that all serotypes of mutans streptococci bacteria are capable to express GTF. The extraction of GTF from two species of mutans streptococci most commonly isolates from tooth samples are *S. mutans* and *S. sobrinus*. The first is more cariogenic than the second because specific cell-surface protein of *S. mutans*aid in its primary attachment to the tooth, while *S. sobrinus* lacks such proteins (Islam *et al*., 2007).

The present study concluded that *S.mutans* (serotype C) is the most isolated bacterial species of mutans streptococci from the human dental plaque. Other serotypes of mutans streptococci bacteria are able of producing two types of glucosyltransferase, extracellular GTF and cell-associated.

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