

THE ROLE OF SOME VIRULENCE FACTORS OF *STREPTOCOCCUS MUTANS* BACTERIA ISOLATED OF PATIENTS WITH DENTAL DISEASES IN HILLA CITY

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ABSTRACT

Streptococcus mutans is considered the major and the etiological agent of human tooth decay. It is an obligate biofilm – forming bacterium, which resides on teeth and forms together with other species, an oral biofilm that is frequently designated as supragingival plaque. *Streptococcus mutans* has virulence factors to help to protect the bacterium against possible defenses and maintain its ecological niche in the mouth. Possible virulence factors include: adhesions, acid production, acid tolerance, production of glucosyltransferase *GTFs*, biofilm formation, capsule, dextranase, and mutacin production, lead to dental infection. This study included (100) samples which were collected from patients suffering from dental diseases, (50) samples

taken from patients infected with dental caries and (50) samples taken from patients with periodontal diseases, who have admitted to a collage of Dentistry in Babylon university and special center of Dentistry. (30) Samples were taken from healthy persons as control, during the period from the first of November 2014 to end of April 2015 in Al-Hilla city. The isolation and identification of *S.mutans* was performed by Mitis- Salivarius Bacitracin agar as a selective medium and biochemical tests. The result showed that 24 (24%) of samples revealed positive growth of *S.mutans* . The virulence factors of *S.mutans* isolated in this study were studied and it was seen that the (58.3%) of isolates were made capsule and (100 %) were its ability to synthesize glucan, (83.3%) of *S.mutans* isolates were able to produce mutacin and (45.83%) of isolates were found to produce dextranase enzyme, while (84%) were able to biofilm formation in addition, all isolates in this study were able to adhere to oral epithelial cells. Finally the most isolates are able to produce many virulence factors involving,

mutacin, glucan as well as adhere to the tooth surface of human (biofilm), dextranase, capsule and adherence ability to oral epithelial cells.

KEYWORDS: Dental diseases, *Streptococcus mutans*, GTFs, Capsule, Dextranase, Biofilm formation, Mutacin production, Adhesive properties.

1. INTRODUCTION

Dental diseases are the one of the most common human population, universal infectious diseases.^[1] These diseases can occur and associated with: main changes of the mouth biology from exogenous origin, such as intake of dietary fermentable carbohydrates or from endogenous alterations like mutating in the integrity of the host defenses.^[2]

Dental caries, also called tooth decay is a major transmissible bacterial infections disease that damage the crystal surface intra enamel, dentine and/or cementum of the tooth by producing organic acid from the cariogenic bacterial metabolism of fermentable sugars. The resulting copious acidic amount in the mouth will affect tooth causing lower the pH to a level where dissolving the minerals of enamel and dentin.^[3]

Other dental diseases are gingival and periodontal diseases induced by dental plaque (accumulation of bacterial layers). Gingivitis is the existence of gingival inflammation with no lostness of connective tissue interrelation while periodontitis includes extensiveness loss of the connective tissue and alveolar bone around the teeth.^[4]

Streptococcus mutans bacteria are the most common important causative agent for tooth decay, because it is adhered and accumulated in large size on dental enamel, plaque formation and localized demineralization of dental enamel.^[5] *Streptococcus mutans* cells are Gram positive, ovoid or spherical in shape, organize in pairs or short chains during the growth of bacteria, non-spore forming, facultative anaerobic, non-motile. It synthesizes and package it in a capsule that is compounded of the polysaccharide dextran, catalase - ve.^[6] Recent studies, report that untreated patients with periodontitis have high recovery rates of *S. mutans* from saliva, tongue dorsum, buccal mucosa.^[7]

Streptococcus mutans produce some virulence factors of help to protect the bacterium against possible defenses and maintain its ecological niche in the mouth. Possible virulence factors include.

Adherence

Adherence factors are called fimbriae which act on agglutinating the bacteria in epithelial cells. Several members of the bacteria have fimbriae that response to adherence to surface of epithelial cells, various strains or species of bacteria may produce different types of fimbriae.^[8]

S.mutans is able to produce biofilm, the development of biofilm occurs in two discriminating phases: during the first, bacterial surface proteins interact with host or bacterial products colonized on the tooth enamel. In the second phase, biofilm shapes as bacteria accumulate by aggregation with the same or other species and producing extracellular poly saccharide (dextran).^[9]

Dextran contains a capsule that attaches to the tooth surface and form biofilm that includes 300-500 bacterial cells (Todar, 2008).^[10] The first step for colonization is the adherence of oral bacteria to the dental enamel. *S. mutans* primary adherence is mediated by a diversity of surface associated protein such as adhesion P1 (also called SpaP or antigen I/II), which binds to the salivary proteins found in the pellicle on the tooth surface.^[11]

Glucosyltransferases (GTFs)

The major mechanism behind sucrose-dependent adhesion is the action of (GTFs) in the synthesis of glucans. The *GTFs* have a sucrose activity that results in cleavage sucrose, in to glucose and fructose.^[12] The glucose part is then added to a growing polymer of glucan. *S. mutans* has three *GTFs* encoded by *gtfB*, *gtfC*, and *gtfD*, harbor three genes encoding *GTFs*. Aggregatively, the *GTFs* synthesize both water soluble and WIGs. The water- soluble glucan (WSG) is prevalently linear polymer linked by alpha 1,6-glycosidic linkages that called dextran, the WIG is prevalently a higher degree of branching polymer linked by 1,3- linkages that called mutan. Both polymers are thought to participate to sucrose dependent colonization and caries, but WIG may be of main importance for smooth surface caries.^[13]

Acidogenicity and Acid Tolerance

Streptococcus mutans ferment abundant different sugars and the metabolize sucrose to lactic acid more rapidly than other oral microorganism.^[14] This metabolic reaction causes the dental plaque acidic in the presence of fermentable carbon source. Until at the low pH acid tolerance of *S.mutans* enable them to continue metabolism.^[15]

Dextran-Hydrolyzing Enzymes

Dextran is homoglycan containing α -D glucose molecule branches and bounded by α -1,3 (water-insoluble) and α -1,6 (water soluble glycosidic bond).^[16] Dextranase are dextran –degrading enzymes, which produce the diverse combination of different carbohydrases. These enzymes have often been classified as exo and endo dextranases based on the mode of action and generally called dextranase.^[17] *Streptococcus mutans* depolymerize the dextran polymer in dental plaque and utilize the dextran as a source of carbon,^[18] Dextranase can break down various microbial dextran sediments; actually that dextran is a component of dental plaque which contributes to the development of dental infection.^[19]

Mutacin Production

Bacteriocin is a peptide antibiotic substance produced by living microorganisms inhibits or kills the growth of other living microorganisms.^[20] *S.mutans* is adapted to the biofilm existence and there is concerted production of bacteriocins along with an increase in competence in high density locations. Bacteriocins are called according to the bacterial species producing them; bacteriocin are manufactured by mutans streptococci is named mutacin.^[9] Theoretically, the capacity to produce broad spectrum mutacins could favor the colonization of producer *S. mutans* in the compound dental biofilm, so increasing the risk of tooth caries.^[21]

2. MATERIAL AND METHODS

2-1 Sample

A total of (100) samples was taken from a patient suffering from dental diseases (50) samples were taken from patients with dental caries (23 males and 27 females), (50) samples were taken from patient with periodontal diseases (15 males and 35 females), and (30) swabs were taken from healthy persons (control) who admitted to a consultant unit of the college of dentistry in Babylon university and also to consultant special center of dentistry in Hilla city. The study lasts five months from the first of November 2014 till the end of March 2015. The specimens were taken by sterile excavators from the middle of the soft dentine lesion in case of carious teeth,^[22] While the plaque and calculi (tartar) were collected by sterile curette and scalar in case of periodontitis and gingivitis.^[23]

The specimen was placed in sterile test tubes containing 1ml normal saline to prevent dryness and stored in a cool box while immediately reaching the laboratory to be dispersed for 30

second by vertex mix. Tenfold dilution was made by normal saline, then inculcated on Mitis-Salivarius-Bacitracin (MSB) agar by sterile cotton swab. ^[24] All plates were incubated anaerobically using anaerobic Gas-Pak System for 24-48hr at 37°C for bacteriological analysis. ^[25]

2-2 Identification of *Streptococcus mutans*

Streptococcus mutans were examined directly and under a dissecting microscope (magnification **x 15**) and detected according to their morphological characteristics on the MSB agar plates, including studies of the colonial characteristics (shape, volume, borders and texture) according to the description cited by, ^[26] and examined by light microscope after staining with Gram's stain according to, ^[27] also **biochemical tests** such as (Oxidase test, Catalase test, Simmon's Citrate test, Motility test, Methyl Red test, Vogues – Proskauer test, Urease test, Carbohydrate fermentation test, and Salt tolerance test (NaCl). *Streptococcus mutans* were examined under microscope by direct smear without staining for detection of bacterial motility ability. ^[28]

2-3 Virulence Factor Tests

2-3-1 Capsule Production Detection

It was accomplished by using the India ink stain to detect the capsule production of *S. mutans*. A single colony of bacterial growth is suspended in a drop of India stain and will mixed, then spreading on glass slides by the spreader. The slide had been dried in the air (room temperature), and examined with an oil - immersion lens of microscope to detect the capsule. ^[29]

2-3-2 Detection of Glucan Production by Glucoamyltransferase

Tested isolation were inoculated on the glucan production medium and incubated at 37°C for up one week. The formation of Complete or partial gel in the medium was observed by kindly tipping the tube back and forth indicating glucan production by GTFs. ^[30]

2-3-3 Dextranase Production Detection

Test isolates were inoculated on dextranase production medium and incubated for approximately 48 hrs. Benedict solution 3ml had been added to this broth and heated in water bath at 100°C for about 20-30 minute. The change in color from blue also brown or purple indicates positive results. ^[31]

2-3-4 Biofilm Formation: Micro Titer Plate (MTP)

The MTP assay described by Christensen *et al.* [32] is most widely used and was regarded as a standard test for the detection of biofilm formation. In the present study, all isolates had been screened for their ability to configure biofilm by a MTP method with a modification in the duration of incubation which was expanded to 24 hrs. Isolate from the fresh agar plate was inoculated in tryptic soy broth medium and incubated for approximately 18 hrs, at 37°C in stable condition and diluted 1 in 100 with fresh medium. Individual wells of sterile polystyrene 96 well-flat bottom tissue culture plate was filled with 0.2 ml of the diluted culture and only broth served as a control for exam sterile and non-specific binding of media. The micro titer plate has been incubated for 18 hrs and 24 hrs at 37°C. After the incubation content of each wall has been kindly removed by tapping the plate. The wells have been washed four times with 0.2 ml of phosphate buffer saline (pH 7.2) to remove free –floating 'planktonic' bacteria. Biofilm is formed by adhesive 'sessile' organism in the plate which has been fixed with sodium acetate 2% and stained with crystal violet (0.1% w/v). Increased stain has been rinsed off by thorough washing with de-ionized water and the plates have been kept for drying. Adherent bacterial cells normally formed on all side wells and were evenly stained with crystal violet. Optical density (OD) of stained adherent bacteria was calculated with a micro ELISA auto reader at a wavelength of 570 nm (OD570). An experiment was performed in triplicate and three times, the data were then averaged, and the results were interpreted according to the table 1 . [33]

Table 1: Classification of bacterial adherence by tissue Culture Plate Method (TCP)

Mean OD values	Adherence	Biofilm formation
< 0.120	Non	Non/ Weak
0.120-0.240	Moderately	Moderately
>0.240	strong	High

2-3-5 Adherence Ability Test

The ability of *S. mutans* to adhere to oral epithelial cell is one of the important virulence properties of these bacteria. This test was done by preparing the Thyoglycolate broth (bacterial broth) and incubated under anaerobic condition for 72hrs. Bacterial broth dilution was prepared by using phosphate buffer saline (PBS) then taken 1.5×10^8 CFU/ml. The oral epithelial cells were prepared by swabbing the epithelial layers of the oral cavity by cotton swabs, then transferred directly into sterile tubes containing PBS (pH 7) after that it were washed with PBS by using a centrifuge (5000rpm for 10 minutes) for three times. Then,

the PBS contains epithelial cells, which had been filtered by filter paper and placed the epithelial cells on the cover slide by pressing the cover on the surface of filter paper then lifted to be dry. Cover slides were placed on sterile a glass plate, then added 5ml of previously prepared bacterial broth, then placed the plate which contains the epithelial cells and bacterial broth on incubator for 1 hour at 37°C. Cover slides were washed with PBS to remove adherent bacteria. The epithelial cells were fixed by ethanol for 15 minutes and then stained with Giemsa stain (30%) for 20 minutes, then washing the cover slides by D.W and lifted to dry by air, then the cover slides were placed on glass slides by inverted position, and then tested under a light microscope.^[34]

2-3-6 Mutacin Production

This method described by Al-Qassab and AL-Kafaji.^[35] Media 1 streak of the test strain by vertical line was done on Blood Tryptic Soy agar and then incubated at 37°C for 48 hrs to permit bacteriocin to spread around the growth line.

- On the next day sensitive or indicator strain has been inoculated on nutrient agar and incubated at 37°C for 24hrs.
- On the third day, the petri-plate cover of the streak plate has been covered by filter paper saturated with chloroform in an upright position.
- Then plate culture was being inverted on its cover for 15 minute. The culture was abraded by sterilizing glass slide into disinfecting vessel, and the plate culture has been exposed to chloroform vapors and then left the plate open for 1 hr to remove the chloroform.
- Inoculated sensitive or indicator strain (which has grown on nutrient agar) had been streaked crossing the original inscribed streak line on tryptic soy agar plate culture and incubated at 37°C overnight. The mutacin production was being abraded as growth inhibition at the medical streak line.

3. RESULTS AND DISCUSSION

3-1 Isolation and identification of *S. mutans*

Colonies of *S. mutans* on the selective MSB agar plates appeared bright blue in color, approximately (1-2mm) in diameter as sphere-shaped or ovoid in form with elevated and convex surface and complied with the agar surface as in a Figure 1.



Figure 1: *Streptococcus mutans* on MSBA

Microscopic Examination *S.mutans* cells were shown G+ ve, circular or ovoid in shape, arranged in short or medium length non-spore forming chains by microscopic examination, and it was non-motile when observed their motility under microscope by direct smear. This result was also reported by Marsh and Martin. ^[2] and Zhu et al. ^[6]

3-2 Biochemical Tests

These consequences of biochemical tests were adopted in the table 2, as a complementary feature of the early diagnosis of *S.mutans*,

Table 2: Biochemical tests of *S. mutans* sequesters in this study

No	Primary tests	<i>S. mutans</i>
1	Catalase test	-ve
2	Motility test	- ve
3	MR test	+ve
4	Oxidase test	-ve
5	Simmon's citrate test	- ve
6	Urease test	-ve
7	VP test	+ve
8	Sugar ferment	
8-1	D-mannitol	+ve
8-2	Glucose	+ve
8-3	Inulin	+ve
8-4	Lactose	+ve
8-5	Raffinose	+ve
8-6	Ribose	-ve
8-7	Sorbitol	+ve
8-8	Sucrose	+ve

+ve: positive result, -ve: negative result, VP: Vogues-Proskauer test, MR: methyl red test.

The results a total of (100) samples, only 24 isolates (24%) positive for *S.mutans*. Fifteen isolates (30%) were taken from dental caries lesions, and nine isolates (18%) were obtained from dental plaque. No isolate of *S.mutans* was isolated from (30) samples of the healthy control, as on table 3.

Table 3: Frequency of *S.mutans* isolates according to the type of dental diseases

Type of dental disease	No.of Samples	<i>S.mutans</i>	
		No	%
Dental caries	50	15	30%
Periodontal diseases	50	9	18%
Total	100	24	24%
Control (healthy)	30	0	0%

This explains that *S.mutans* represent the main causative agent of dental diseases, particularly in dental caries and minor grade in the periodontal diseases also not found in healthy individuals. These results agree with those obtained by Shimotoyodome et al. [36] and Todar. [10] while Contardo et al. [37] who reported that untreated patients with periodontitis have recovery rates of *S. mutans* from saliva, tongue dorsum, buccal mucosa. *S.mutans* bacteria, according to these results, played direct and important role in pathogenesis of dental caries as recorded by other studies such as Lynch at al. [38] This determination was also well-matched with results obtained by Kolenbrander. [39] and Aas et al. [40] who described that *S. mutans* was the major causative agent for dental caries and play an indispensable part in the development of other kinds of microorganism via the manufacture of suitable environment during the buildup of oral bacteria on the tooth surface, partly as a result of *S. mutans*, which is able to produce dextran substance of the dental plaque by its GTFse enzyme.

3-3 Detection of several virulence factors for *S. mutans*

3-3-1 Capsule production

This study was explained that the *S.mutans* was able to capsule production as shown in Figure 2, it was shown about (58.3%) of isolates were made capsule production. This result had been nearly in agreement with the results obtained by Hardie. [41] The capsule of *S.mutans* composed of extracellular sticky dextran polymers, which is able to initial attachment of the bacterial cells to the tooth. [10]



Figure 2 : Illustrated capsule of *S.mutans* (100x magnifications).

3-3-2 Glucan production

One of the first important virulence factors of *S.mutan* was able to produce glucan as shown in Figure 3. About 24 (100 %) of isolates were its ability to synthesize glucan by GTF, when inoculated on glucan medium for seven days at 37°C. These results were in agreement with the results obtained by Kuramitsu and Wondrack. ^[42] who had indicated that the *S.mutans* created absolute percentage of glucan by GTFB enzyme. The production of glucan polymers from sucrose, is made by the GTF supply binding site of bacterial linkage on the tooth enamel and also for the accumulation with some bacterial oral cavity. It can synthesize bulky amounts of sucrose by GTFs. Loesche. ^[43] establishes that the large amounts of glucan lead increase amounts of organic acid (lactic acid) by the fermentation of sugar constitute main virulence factors in the causation of dental caries which is shown beyond misgivings.

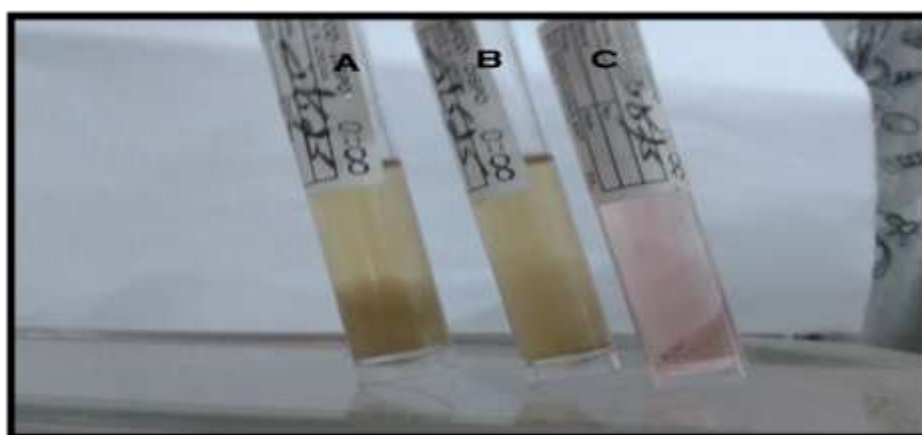


Figure 3: Glucan production by *S. mutans* (A) -Glucan production after seven days, (B)- Glucan production after five days, (C) –negative result.

As declared above, *S. mutans* interceded damage to the teeth is associated nearly wholly with bacterial metabolism. Initially, the virulence characteristics of Mutans streptococci were identified, following contrast to the other streptococci in the mouth. One of the first virulence factors identified by this style in vitro was the ability to colonize of smooth surfaces. [44] because of the ability of making extracellular polysaccharides from sucrose. *S. mutans* synthesizes α -1,3 and α -1,6 linked glucan polymers by using sucrose through the action of GTFs, encoded by the *gtfB*, *gtfC* and *gtfD* genes. GTFB makes mostly water insoluble glucans (rich in α -1,6- linked glucose) called mutan, GTFD makes predominantly soluble glucans called dextran, whereas GTFC makes a mixture of soluble and insoluble glucans. [45] Water-insoluble glucans are sticky nature accelerates the linkage of bacteria to the teeth and resists detachment by mechanical forces.

3-3-3 Mutacin production

Initial studies about the detection of mutacin were done and the results had been shown that 20(83.3%) of *S. mutans* isolates were able to produce mutacin as illustrated in Figure 4.

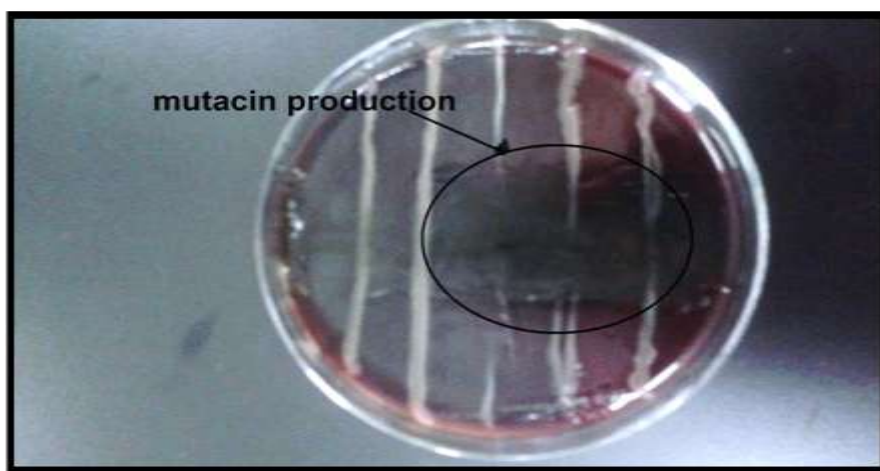


Figure 4: mutacin production by *S. mutans* on tryptic soy blood agar.

These results were similar to those obtained by Gronroos *et al.* [46] who showed about (88%) of isolates produced mutacin and another study by Kamiya *et al.* [47] who was found that the (83%) of isolates were able to make mutacin against one or more indicator strains. The ability of *S. mutans* to produce mutacin may consult ecological advantages of creating strains on other sensitive bacteria in varied bacterial populations such as dental caries and biofilm. [48] Another latent virulence factor of *S. mutans* may be their production of mutacins. Mutacins (bacteriocins) are ribosomally synthesized peptide antibiotic, which are antimicrobial

activities against closely linked other competing species in the dental plaque.^[49] *S.mutans*, two groups of mutacines have been classified: lantibiotic peptides are post translationally modified to represent by mutacin I, II and III and, nonlantibiotic unmodified mutacin, represented by mutacin IV. The lantibiotics have a broad spectrum, including most of G+ve bacteria, while the nonlantibiotics have a narrower spectrum. They kill commonly streptococci belonging to *S.sanguinis* and *S.mitis* groups.^[50]

Bacteriocins activity can help *S.mutans* contest for limited nutrients presented in its environmental niche. *S.mutans* can not only diminish its competitors for food, but also confirm that high amounts of heterologous DNA are present during development of the genetic competence, by concurring bacteriocins produced by population density. As well as producing bacteriocins, *S.mutans* is shown to produce bacteriocin- immunity proteins, which is able to modulate the sensitivity to antimicrobials in *S.mutans*.^[51]

According to Gronroos et al.^[52] the antagonism probably happens in the dental enamel and may be important for the stability of *S.mutans* in a situation, mutacin production by *S. mutans* which may encourage the transmission of bacteria from mother to child and may have an important role in the colonization of the species in the oral cavity because of their selective antagonism mechanisms. Theoretically, the ability of *S. mutans* to produce broad spectrum mutacins could favor the colonization of produce these bacteria in the complex dental biofilm, so it increases the risk of caries.

3-3-4 Dextranase production

The isolates of *S.mutans* were subjected for its ability to produce dextranase enzyme after growth in dextranase production medium containing 10% sucrose. After (48 hrs) of incubation, 3ml of Benedict solution was added, only 11 (45.83%) of *S.mutans* were found to produce dextranase enzyme as shown in figure 5. This result was similar to other studies that observed *S.mutans* are the main producers of dextranase enzyme Igarashi et al.^[53] Khalikove et al.^[54] who had shown that the bacteria has the ability to create dextranase enzyme which decompose the dextran by hydrolyzing α -1,3 and α -1,6 glycoside linkages that carry the glucose polymer in dextran molecules.

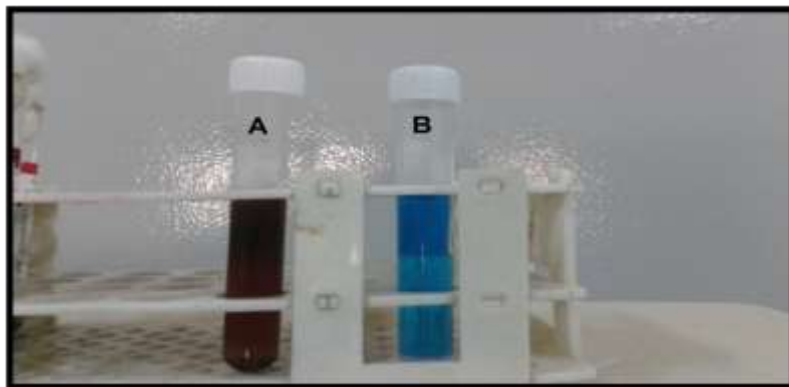


Figure 5: dextranase production by *S.mutans*, A:positive result. B: negative result

Dextran is the important extracellular polysaccharide of bacteria. It can produce, from dietary sucrose encouragements, dental plaque formation that leads to the development of dental caries and periodontitis. ^[19] When the exogenous source of sugar (dietary sucrose) is reduced to stimulate the ability of *S.mutans* to produce dextranase. Cariogenic *S.mutans* will produce this enzyme to depolymerize the dextran polymer in dental plaque in to glucose molecules and use the molecules as an exclusive source of carbon for nutrition requirement ,thus *S.mutans* still produce lactic acid even when the fermentable sugar was not taken. This organic acid causes dissolving of the enamel and dentin under low pH condition , thus *S.mutans* will continue producing caries even after thorough cleaning of teeth. ^[10]

3-3-5 Biofilm formation

It is another virulence factor for *S.mutans*, it's the ability of generating biofilm was shown in Figure 6 .The results had been investigated that 12 (50%), 9 (37.5%) of isolates were formed strong and moderate biofilm respectively, and, 3(12.5%) were weak biofilm formation, when bacteria grow on tryptic soy broth in polystyrene cell culture plate,as illustrated in table 4.

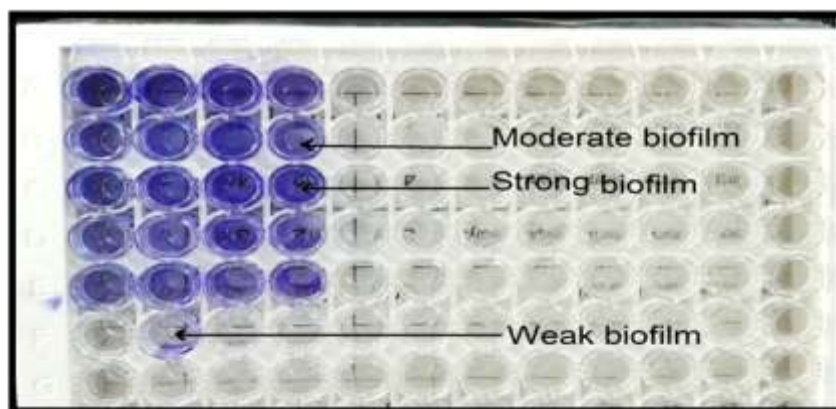


Figure 6: Biofilm formation by *S.mutans*.

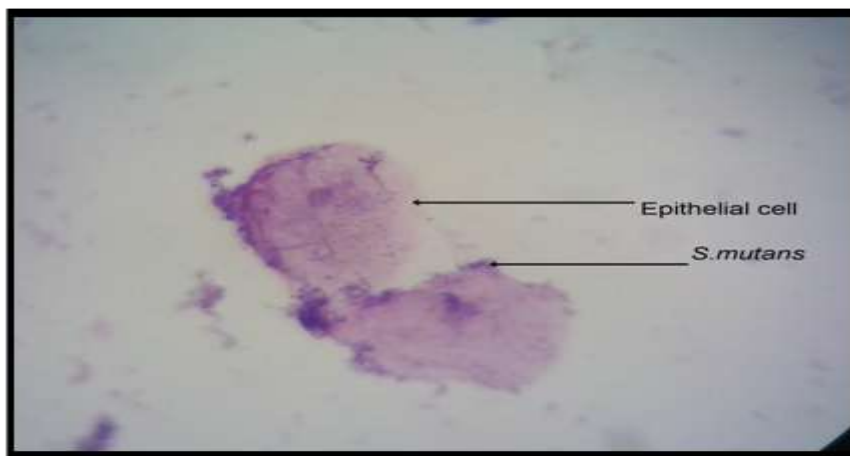
Table 4: Means formation of biofilm in *S.mutans*.

Bacterial Isolates (no)	Biofilm formation			
	Strong No %	Moderate No %	Weak/none No %	Biofilm formation No %
<i>S.mutans</i> (24)	12 (50%)	9 (37.5%)	3 (12.5%)	21 (87%)

These results were nearly similar to those obtained by Decker et al. ^[55] who had found that the (84%) of *S.mutans* isolates were able to biofilm formation. Biofilm cells growing on surfaces display properties different from those of planktonic cells, for example enlarged resistance to biocides and antimicrobial agents. ^[56] also it has been progressively concerned as a key pathogenic factor in biomaterial infections. ^[57] Biofilm matrix is extracellular polysaccharides, which are produced by *S.mutans* from dietary sucrose metabolism and facilitates the association of mutans streptococci with dental plaque and the colonization of dental plaque by the bacteria which plays a causative role in dental caries. ^[58] The formation of sucrose –dependent adhesion biofilm plays a central part in the ability to colonize and accumulate *S.mutans* on the tooth surface .Water-insoluble glucans (WIG) synthesized by (GTFs) constitute the principal role of the matrix of sucrose –dependent biofilm and supply binding sites for *S.mutans* for colonization and adherence. ^[59]

3-3-6 Ability of adherence

The first step of the colonization of opportunistic bacteria can be adhered on host cells. The ability of *S mutans* to adhere to epithelial cells of the oral cavity is one of the important virulence factors of the bacteria. The result was observed that all *S.mutans* isolates in this study were able to adhere oral epithelial cells. Notice in Figure 7.

**Figure 7: Ability of *S.mutans* to adhere to epithelial cell (100x magnification).**

when the oral epithelial cells were placed on cover slide and put in bacterial broth, then stained by giemsa stain. This result was compatible with results obtained by Jacobo et al. [60] who found that the ability of *S.mutans* to adhere to polymerized material more than *Lactobacillus gasseri*. Inactivated *S.mutans* retained the capacity to adhere to the polymerized material because of the streptococci capsular polysaccharides.

The attachment of *S.mutans* to oral surface, which are usually coated with salivary molecules, is an initial event in the development of dental caries. One of the first oral streptococcal surface protein antigens is shown to be adhesion which is antigen I/II. [61] Salivary agglutinin is a glycoprotein, which mediated the adhesion and aggregation of *S.mutans* by the cell wall-associated adhesion P1 (member of the Ag I/II family of cell surface proteins. [62]

Bacterial fimbria mediated interaction is postulated as a mechanism of adhesion of oral bacteria to the tooth surface. The specificity displayed during interaction of bacterial adhesin and host cells is associated with antibody – antigen – specific recognition and explained why *S.mutans* is more copious in dental plaque than other site of oral cavity. [63]

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