



Investigation of *Streptococcus mutans* isolated from dental caries patients

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Summary

Introduction: The clinical specimens (100) were collected from patients suffering from dental plaque infectious during the period from October (2012 to March 2013) in Najaf City.

Methodology: *Streptococcus mutans* isolates were detected by cultural and biochemical tests as well as API 20strep, then confirm the *gtfB* gene by PCR technique.

Result: *Streptococcus mutans* were recorded (40%) among *Mutans Streptococcus* from total isolates. The virulence factors of bacteria were detected and the results showed that all isolates were produced glucan, biofilm and Dextranase 40(100%) and varied produce hemolysin 30(75%). Also, 20-25(50-62.5%) of *Streptococcus mutans* has the colonization factor antigen III, I respectively and 15 (37.5%) of isolates are mutacin production. Finally, Molecular study by PCR technique was used for detection *gtfB* gene, it was found the *gtfB* gene predominance in 22 (55%) of clinical isolates.

Conclusion: The study showed that *Streptococcus mutans* have *gtfB* gene which responsible for the pathogenicity of *Streptococcus mutans*.

Introduction



Streptococcus mutans are gram-positive cocci, nonmotile facultative anaerobic microorganism which can metabolize carbohydrates and are considered to be the principle etiological agent of dental caries (Tanzer *et al.*, 2001). The cariogenicity of this bacterium is associated with various factors including dextran, production of high concentration of acid in the plaque and glucosyltransferase activity.

The *Mutans streptococci* use sucrose to produce extracellular glucan, a water insoluble polysaccharide, which enables the bacteria to attach to the tooth surface and also protects them from external factors such as mechanical disruption, salivary clearance, and antimicrobial substances (Tinanoff *et al.*, 2002). Equally important is the ability of *Mutans streptococci* to both produce acid (acidogenic) and survive in an acidic environment (aciduric), properties that enable them to exhibit high pathogenicity (Aas *et al.*, 2008).

Finally, *Mutans streptococci* can survive when external carbohydrates are not present due to their ability to store intracellular (Tinanoff *et al.*, 2002). The Water insoluble glucans are significant constituent of dental plaque biofilms that facilitate adherence and accumulation of *S. mutans* and other oral bacteria. The biofilm formation is influenced by the amount of glucosyl transferase produced by *S. mutans* are the most common pathogens isolated from human dental plaque and their prevalence has been reported in several epidemiological studies (Straetemans *et al.*, 1998; Okada *et al.*, 2005).

Streptococcus mutans is very important to study, not only because virtually everyone in the world carries it, but also it has various symptoms that affect our daily lives. As the bacteria develop in the mouth, they



cause tooth destruction ,impaired speech ,difficulty of chewing , multiple infection , Psychological problems such as poor social interaction ,concentration problem , etc (Philip *et al*,2009). Because, the incidence of infection caused by *Streptococcus mutans* in dental caries samples, the study aimed to identified the bacteria and study related to its ability to produce the virulence – associated properties , as well as the Glucoasyltransferase genes detected by PCR.

-Methodology:

- Identification of bacteria

A single colonies were isolated from primary positive cultures (on specific media) and identified by morphological , biochemical tests and API 20 strep (BioMerieux , France) according to the criteria of (Holt *et al*, 1994; Collee, *et al*, 1996; MacFaddin ,2000) .

-Detection of virulence factors :

Hemolysin production was detection according to method of (Collee *et al*, 1996). Capsule was stained according to method of (Bottone *et al*,1998). Colonization Factor Antigen (CFA) (Ofek *et al.*, 1977). bacteriocin production detected by cup assay method was carried out (AL-Qassab & AL- Khafaji,1992). Biofilm production detected by Tissue culture plate method (Christensen *et al*,1985) .Dextranase production was detected according (Staat *et al*,1973).Glucan production was detection according to method of (Emilson & Bratthal,1976).

-Detection of virulence gene. The PCR assay was performed to detect the(gt**fB**) gene for confirmation the identification of *Streptococcus*



mutans and to detect the virulence factors genes , These primers synthesized by Alpha DNA.F gtf-B 5-ACTACACTTTCGGGTGGCTTGG-3 Reverse gtf-B 5-CAGTATAAGCGCCAGTTTCATC-3(company, Canada).

-The results

-*Streptococcus mutans* characterization

A total 100 samples were collected from teeth and round teeth of patients suffering dental carier .The samples are activated in BHI Broth media at 37°C for 18-24h, then, they were incubated on selective media such as blood agar ,crystal violet blood agar and tryptone-yeast extract cysteine with sucrose and bacitracin (TYCSB) at 37°C for 18-24h in anaerobic condition .The colonies are gray or white color on blood agar and crystal violet blood agar with diameter ranged from (0.5-1.0) mm in diameter α -hemolytic colonies and sometime tending to adhere to the surface of the agar .

The colonies of *S.mutans* in (TYCSB) medium appeared white color colony , 1-2 mm in diameter, with smooth surface with entire edge, dry and adherent. Microscopic examination revealed that *streptococcus mutans* is a gram positive cocci occurs in pairs or in short- or medium-length chains, with capsules. The *Streptococcus mutans* isolates are characterized by their ability to ferment glucose and lactose without H₂S and gas formed on kligler iron agar (Acid/Acid), it produces (Acid) yellow color top and bottom (acidic) yellow color but without H₂S and gas ; it gave a negative result to ,catalase , oxidase ,simmone citrate tests, motility and urease, whereas, it gave a positive result to, Vokes Proskeur and methyl red test.Also, differential *Streptococcus mutans* on the other

bacteria by biochemical tests and Epi 20 strep as index (3). It has been shown that *Streptococcus mutans* isolates do not grow in 6,5%NaCl concentration and pH were 3.5 to 6.5.

The results demonstrate that (40/100) and isolates are positive in identification by API20Strep, as shown in Figure (1).The identification percentage is (id% = 95), and the rest (60) strains gave negative for *Streptococcus mutans* identification.

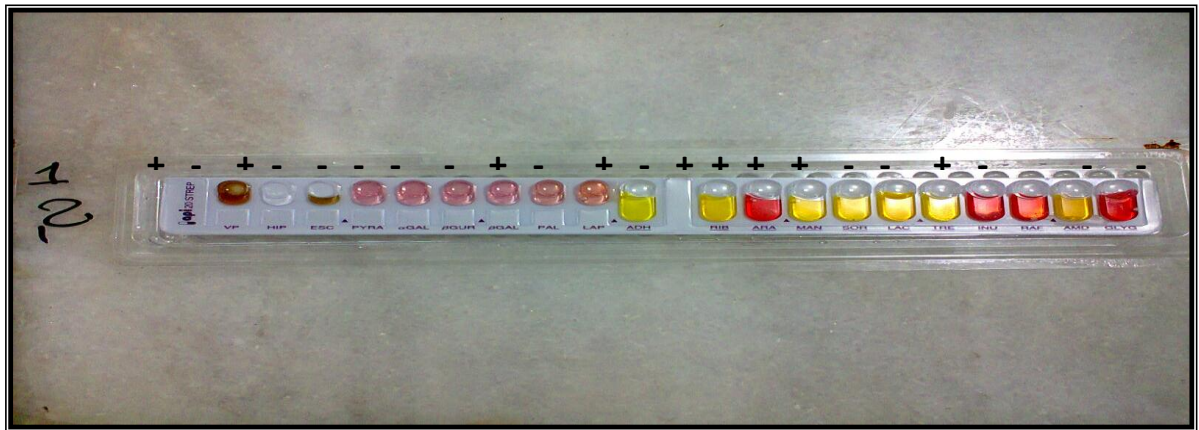


Figure :(1) The Identification of Api20 strep for *Streptococcus mutans* .

-Virulence factors determination:

The results of virulence factors of *Streptococcus mutans* isolates, show that the 30(75%) from isolates of *Streptococcus mutans* were gave positive for heamolysin production , (α -heamolysin), when they were cultured on blood agar medium. Also 20(50%) from isolates were capsulated.



. The 40(100%) from *S.mutans* isolates had the ability to produce glucan by glucoasyltrancferase , when inoculated on glucan medium for week at 37°C. and 15(37.5%) of *S mutans* isolates had the ability to produce mutacin when inoculated on blood tryptic soy agar for 48hrs at 37°C,a 40(100%) from isolates *S mutans* had the ability to produced dextranase ,. A total of *Streptococcus mutans* isolates were for their ability to produce biofilm (20)50%,(15)37.5%) were formed strong and moderate biofilm respectively, 5(12.5%) were weak biofilm formation, .The 25(62.5%) of isolates which had ability to produce CFA / I , while the ability of isolates for produce CFA /III were less 20(50%) , whereas CFA/II do not produce this factor, as table (1).

Virulence factor of <i>str.mutans</i>	No. and% of positive result	No. and % of negative result
Haemolysin	30(75%)	10(25%)
Capsule	20(50%)	20(50%)
Glucan	40(100%)	0(0%)
Mutacin	15(37.5%)	25(62.5%)
Dextranase	40(100%)	0(0%)
Colonization factor antigenI	25(62.5%)	15(37.5%)
Colonization factor	0(0%)	40(100%)

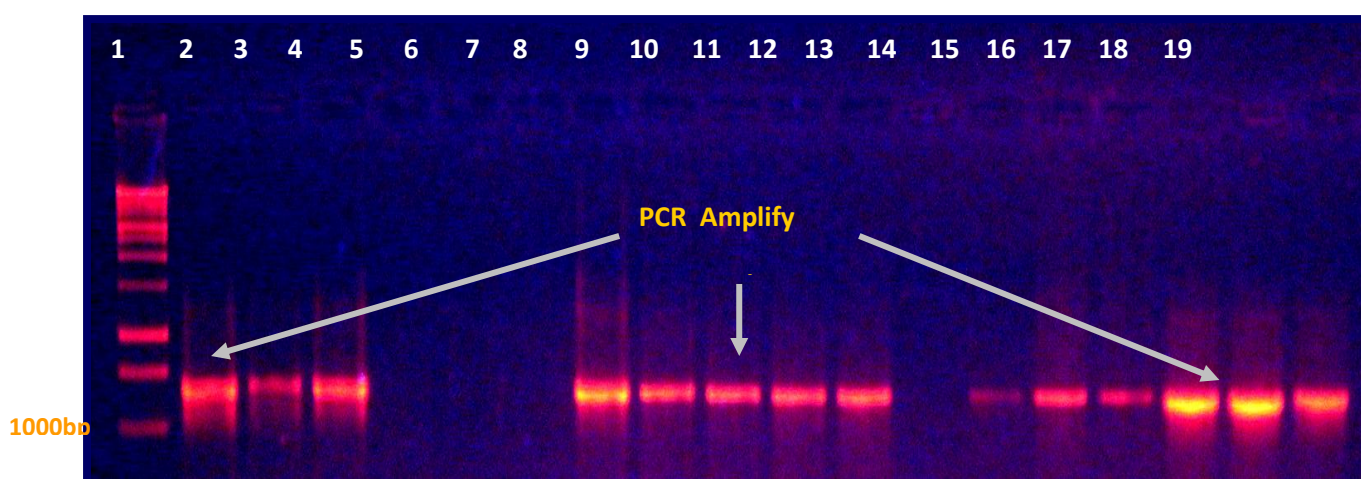


antigenII		
Colonization factor antigenIII	20(50%)	20(50%)
Biofilm formation	40(100%)	0(0%)

Table (1):Virulence factors of *Streptococcus mutans*

-Detection of Glycoasyltransferase Gene (*gtfB*)

Polymerase chain reaction technique has been used to amplify Glycoasyltransferase gene(*gtfB*) from genomic DNA of all *Streptococcus mutans* isolates . Isolates with specific forward and reverse primer ,which responsible for glucoasyltransferase enzyme. The results were shown in Fig(2) .





**Figure (2) :Agarose Gel Electrophoresis (1%) of PCR Amplified of
(*atfB*) gene (497) bp of *Str. Mutans* isolates for (55) min at 100 Volt**

Lane (1) Marker (1kb DNA ladder).

Lane(2) (2, 3, 4, 8, 9, 10,11,12,14,15,16,17,18,19) were positive result

Lane(3) (5,6,7,13) were negative result

- The discussion

-*Streptococcus mutans* characterization

Streptococcus mutans appear as pairs or short chains in microscope examination.. The biochemical tests of *streptococcus mutans* involved its ability to ferment lactose ,sucrose ,mannitol , sorbitol , glucose , inulin ,raphanose, ribose sugar and grow (Acid/Acid) on kligler iron agar and negative for simmone citrate,urease, oxidase ,catalase test



while positive for Voges Proskeur test and methyl red, and the bacteria is not tolerant to NaCl concentrations at (6.5%), (Beighton *et al*, 1991; Hardie, 1986). API20strep system is used to confirm identification of *streptococcus mutans*.

The present study identifies some virulence factors associated with pathogenicity of *Streptococcus mutans*. It is observed that 30(75%) from isolates for *streptococcus mutans* produce haemolysis which causes partial hydrolysis of RBCs on blood agar, (Clarke, 1924). It was observed that 20(50%) isolates of *Streptococcus mutans* were capsule produced (Hardie, 1986). According to the results all isolates of *streptococcus mutans* were to produce glucan 40% and 15(37.5%) mutacin. The dextranase is important virulence factors for *streptococcus mutans* bacteria and all isolates of *Str. mutans* to produce Dextranase. *Streptococcus mutans* produce 40(100%) from Dextranase when they grow on Dextranase medium. The study demonstrates that *streptococcus mutans* are able to produce (95%) biofilm. It was appeared that the 20(50%) and isolates contain 25(62.5%) CFA/III CFA/I (Adegbola & Old 1983; Keller *et al.*, 1998).

The results show that the glucoamyltransferase (*gtfB*) gene in this study were (22%) of isolate as shown in figure (2). The *gtfB* gene is observed as responsible for disease occurrence as caries. The Glucosyltransferase B is an enzyme produced by *Streptococcus mutans*, which catalyzes synthesis from sucrose of insoluble glucans that provide support to the biofilm. It is one of the main virulence factors in the generation of dental caries.



The *Mutans streptococci* use sucrose to produce extracellular glucan, a water insoluble polysaccharide, which enables the bacteria to attach to the tooth surface and also protects them from external factors such as mechanical disruption, salivary clearance, and antimicrobial substances (Tinanoff *et al.*, 2002).

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