Demonstration Of Altered Colony Morphology Of Mutans Streptococci and their Role in Cariogenicity

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الملخص

تكون مجموعة المسبحيات Mutans Streptococci مستعمرات مختلفة في المظهر الخارجي على الأوساط الغنية بالسكروز مثل وسط Mitis Salivarius Agar(MSA). يعزى هذا الاختلاف إلى تباين قدرة الأنماط المختلفة لهذه البكتريا على إنتاج المواد الخارجية على سطح الخلايا مثل السكريات المتعددة (glucan). تم الحصول على ١٠٠ عينة لعاب لأشخاص من كلا الجنسين وبأعمار ٧-٥٢ سنة. أظهرت النتائج أنماط عديدة ومختلفة من حيث شكل المستعمرات وبنسب متفاوتة، ولقد كان للشكل الخشن Rough colony morphology للمستعمرات النسبة الأعلى للعزلات المحلية (٣٨%). كما أظهرت ٥٦%من العينات نمط واحد من بكتريا Mutans Streptococci ، ٤٠% منها أكثر من نمط و أخيراً، ٤% من العينات كانت سالبة لهذه البكتريا. معدل قيمة DMFT (Decayed teeth + Missed teeth + Filled teeth) للعينات ذات النمط الواحد والعينات التي تحتوي على أكثر من نمط كان ٤ و٥ على التوالي. معدل الأسنان المتسوسة (Decayed teeth) كان ٢,٨ و ٣,٦ على التوالي مما يدل على ان الأشخاص المصابين بأكثر من نوع من Mutans Streptococci هم أكثر عرضة للتسوس. تم اختيار ٨ عزلات موجبة لاختباري تخمر المانيتول و كاشف 2,3,5-Triphenyltetrazolium Chloride لإجراء اختبار الالتصاق على الأسطح الملساء في وسط Tryptic Soy Agar + 2% sucrose حيث أظهرت العينات درجات متفاوتة من الالتصاق.

Abstract

Different colonial variants of Mutans Streptococci are observed on sucrose containing media such as Mitis Salivarius Agar (MSA). Variation in colony morphology seems to be related to synthesizing cell surface materials, namely; the glucan capsule. Saliva samples from 100 subjects of both sexes aged from 7-52 years old were enrolled. Mutans Streptococci (MS) with various colony morphology and various degrees of adherence were demonstrated in the present study. The incidence of rough colony morphology was shown to be 38% of the local isolates, in addition to that 56% of the saliva samples harbored one strain of Mutans Streptococci, 40% of them had more than one and 4% were negative. The mean DMFT/dmft (Decayed teeth + Missed teeth + Filled teeth) value was 4, 5 respectively. The mean decayed teeth for samples with one strain was 2.8 while samples with two or more strains had an average of decayed teeth 3.6. which indicates that subjects harboring more than one strain have a higher incidence of dental caries than those with one strain. Eight S*treptococcus mutans* isolates (Mannitol and 2,3,5-Triphenyltetrazolium Chloride positive.) showed various degrees of adherence which is known to be an important virulence factor of this microorganism.

Introduction

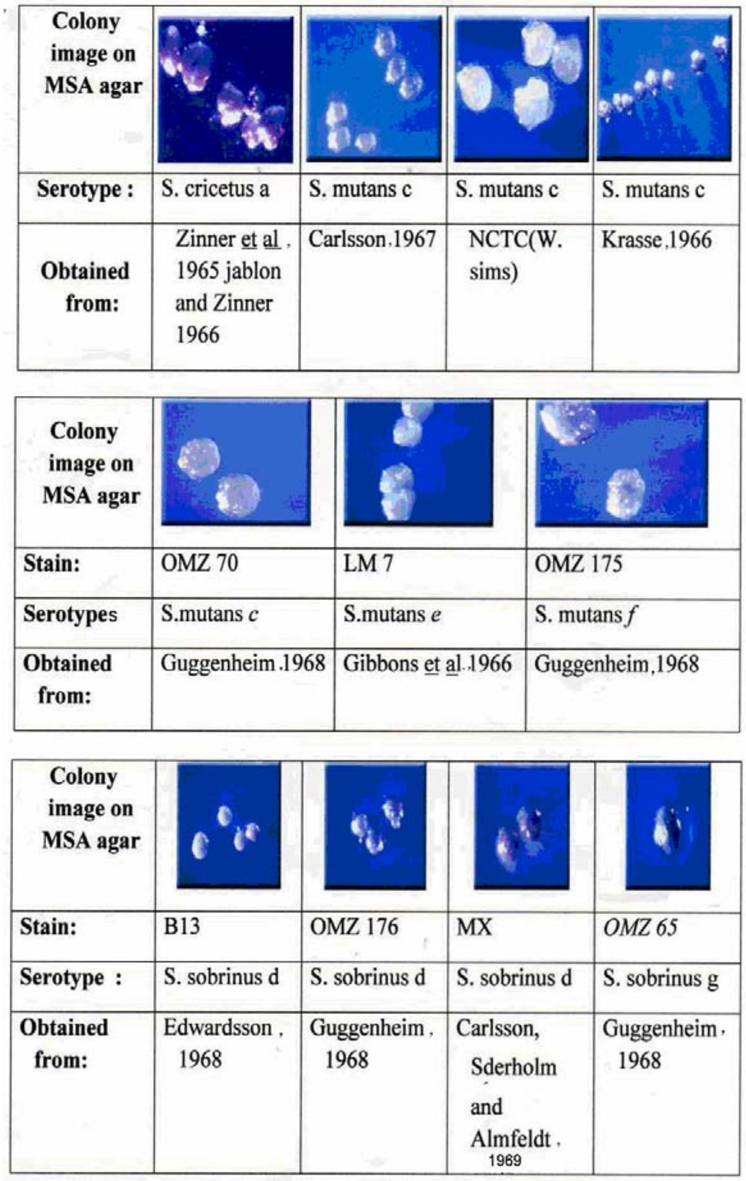
The genus Streptococcus (phylum: Firmicutes, Order: Bacillales, and Family: Streptococcaceae) can be divided into seven groups, Pyogenic, Sanguis, Mitis, Mutans, Salivarius, Anginosus, and Bovis group. The cariogenic organism *Streptococcus mutans* have been divided into serotypes. They have subsequently been elevated to species status and now comprise the "mutans group" of oral streptococci (1).

An important characteristic of this type is the formation of zoogleal colonies on sucrose agar; presumably, this is caused by large amounts of insoluble dextranlike polysaccharides (glucan). Its glucan capsule is synthesized by a class of extracellular and cell-wall associated enzymes called glucosyltransferases and fructosyltransferases. The action of these enzymes on dietary sucrose creates a branched, insoluble glucan matrix that specifically interacts with the tooth surface and with receptors on the cells. Subsequent formation of acids from intracellular glycogen stores in *Streptococcus mutans* and other organisms leads to formation of dental caries (1). Cariogenic bacteria is known to synthesize two types of glucan; water-soluble and water-insoluble glucans, through the latter, a more tenacious attachment to tooth surfaces is achieved. Extracellular dextrans (glucans) produced from sucrose by microorganisms found in dental plaques appear to play at least two roles in the formation of dental caries; first, the cariogenic potential of these microorganisms is dependent on the production of dextran which can initiate cell aggregation and plaque formation (2-5). Second, glucans have been theorized to be important as a part of the stable intracellular plaque matrix (2, 6, 7).

Different colonial variants of this organism are observed occasionally on sucrose- containing media such as Mitis Salivarius agar. Such colonial variants are converted to another morphologic forms by implantation in the mouths of animals and they maintain the ability to induce caries (8). Kondo (9) has shown that small, rough colonies always were derived from mucoid colonies on TCY agar, and that such rough forms usually retained their morphologic form on the agar plate, even after several streaking. Furthermore, in contrast to the original mucoid type strain, the rough variants were almost unable to form artificial plaque on the glass sticks or wire in the presence of sucrose. Variation in colony morphology seems to be related to variation in synthesizing cell surface materials.

Various colony morphology of Mutans Streptococci by some authors, (10) are shown in (figure 1):

Below are some various colony morphology of Mutans Streptococci by some authors, (10) (figure 1):



Colony image on MSA agar				
Serotype :	S. cricetus a	S. mutans c	S. mutans c	S. mutans c
Obtained from:	Zinner et al . 1965 jablon and Zinner 1966	Carlsson.1967	NCTC(W. sims)	Krasse.1966

Colony image on MSA agar			
Stain:	OMZ 70	LM 7	OMZ 175
Serotypes	S.mutans *c*	S.mutans *e*	S. mutans *f*
Obtained from:	Guggenheim.1968	Gibbons et al.1966	Guggenheim,1968

Colony image on MSA agar				
Stain:	B13	OMZ 176	MX	*OMZ 65*
Serotype :	S. sobrinus d	S. sobrinus d	S. sobrinus d	S. sobrinus g
Obtained from:	Edwardsson , 1968	Guggenheim . 1968	Carlsson, Sderholm and Almfeldt . 1969	Guggenheim . 1968

Figure 1: Colony morphology of typical mutans streptococci

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The current study demonstrates various colony morphologies of local strains of Mutans Streptococci isolated from samples taken from Mosul population, biological characterization and their *in vitro* adherence to smooth surfaces.

Materials and methods:

Saliva samples were collected from 100 subjects aged (7-52 years) and both sexes. DMFT /dmft indices reveal the extent to which dental care have damaged a person's teeth, thus it serves as an indicator of dental health. It was recorded by a dentist using a probe and a mirror at daylight. DMFT /dmft is the sum of (decayed untreated teeth +missed teeth+ filled teeth).

All samples were cultured immediately after serial dilution on Mitis Salivarius Agar (MSA) and incubated for two days before colony examination (using a magnifying glass 10x).

A method for identification of *Streptococcus mutans* colonies growing among other oral streptococci on MSA has been developed (13), wherein colonies of *Streptococcus mutans* stain dark pink when sprayed with solution of mannitol or sorbitol and 2,3,5- triphenyltetrazolium chloride, while colonies of the other strepto cocci remain blue (figure 2).

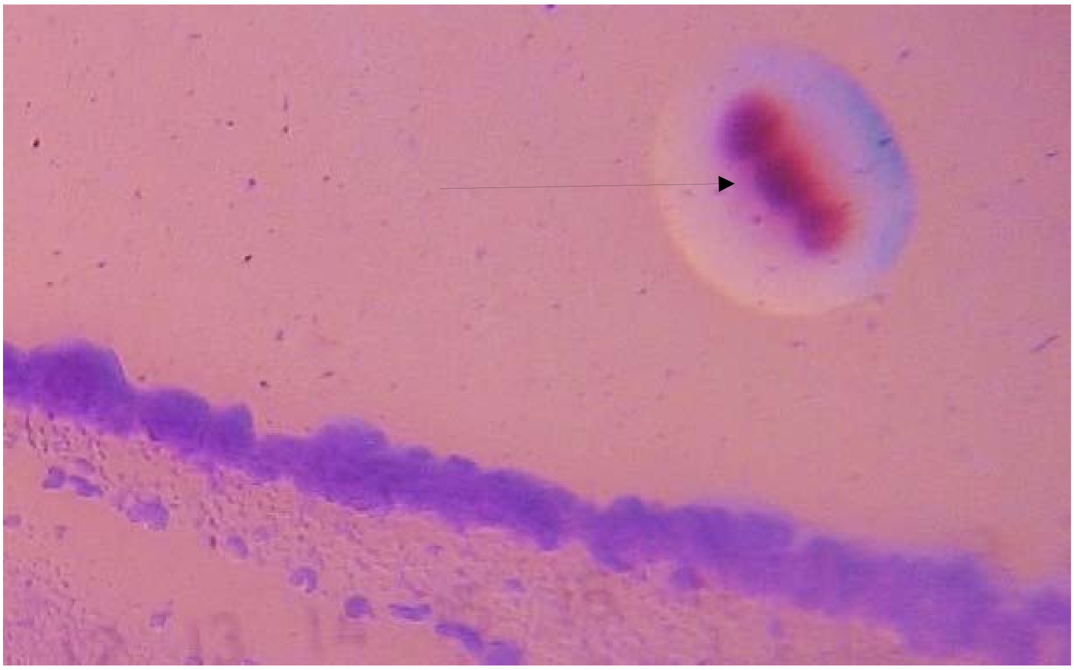

Figure 2: Colonies of *Streptococcus mutans* appear dark pink when stained with 2,3,3-triphenyltetrazolium chloride.

Adherence assay: glass screw-cap tubes containing 20 ml of tryptic soy broth supplemented with 2% sucrose were inoculated with an overnight culture of MS strains and were grown in an anaerobic jar for 18 hours at 37°c. Culture fluid was poured off and the tubes were rinsed three times with 5 ml fresh tryptic soy broth. Adherent cells were stained by filling the tubes with a 10% aqueous solution of crystal violet and allowing them to stand for 1 min. the dye was decanted and the tubes were rinsed three times with distilled water(14). Strains were scored for adherence on a scale of 0 to 4+.

Results and discussion :

Strains of viridians streptococci originally classified as Mutans Streptococci are now separated into at least 7 species and 9 serotypes on the basis of physiological reactions, Serology and DNA hybridization. Identification of *S. mutans* and *S. sorbinus* on selective media such as Mitis Salivarius Agar has been performed using colonial morphology(15,16). Local strains of Mutans Streptococci in the present study have demonstrated various colonial morphology on Mitis Salivarius agar as shown in (figure 3) below.

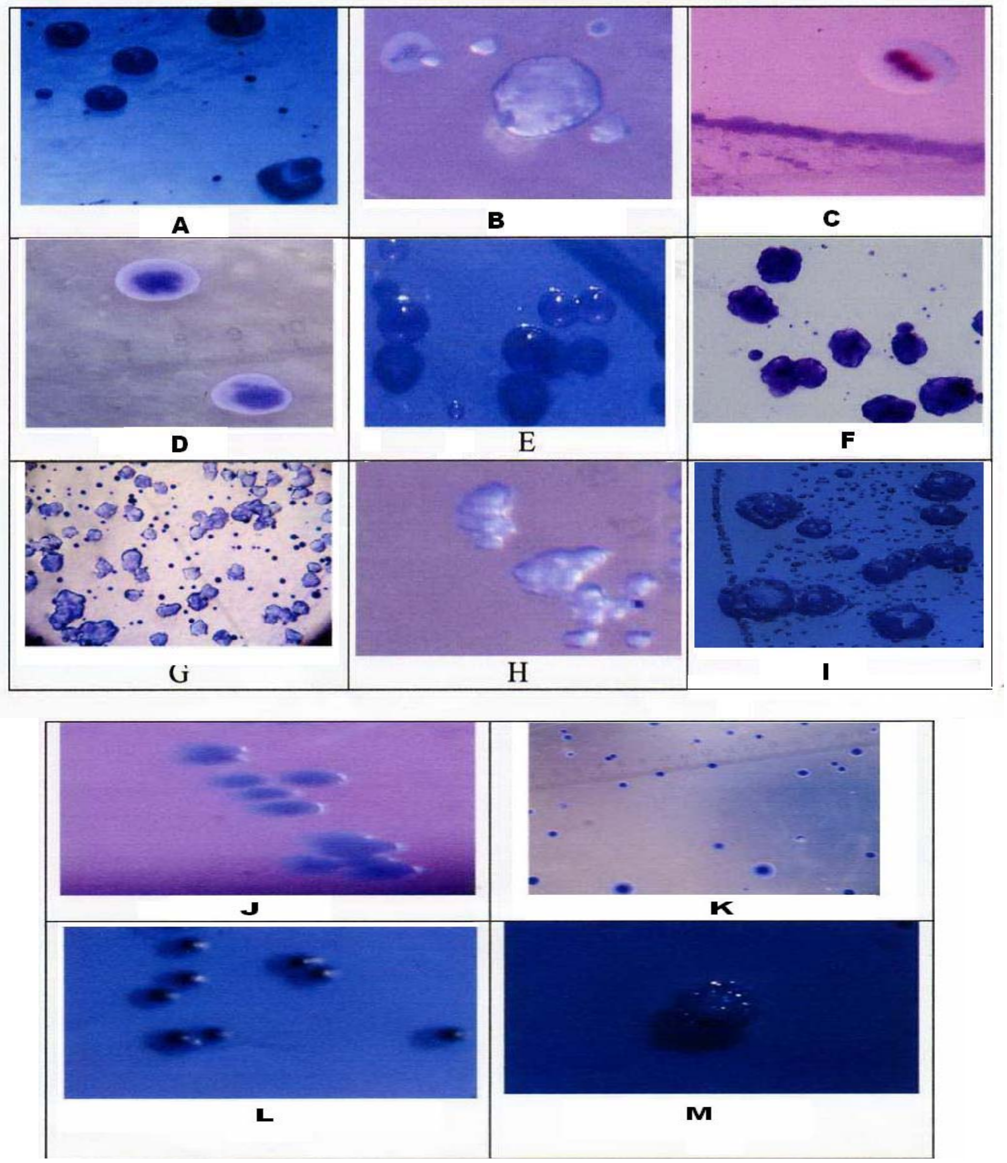

Figure 3: Colony morphology of local Mutans Streptococci (10x).

Results also showed that the percentage of strains isolated were as follows: the rough, raspberry (H, M) was 38%, (K) 35%, (A) 29%, (B) 17%, (E) 17%, and (L) 3%. Epidemiological studies in children and young adults in various geographic areas have revealed some differences in the predominant species and serotypes, but in north America and Europe the incidence of *S. mutans* (serotype c,e and f) has been approximately 80%, *Streptococcus sorbinus*, (serotype d and g) and *S. cricetus* (serotype a) are the next most prevalent species (17). serotype c is the most frequently isolated organism from Japanese children (18).

The prevalence of Mutans Streptococci was determined in the salivas of 100 individuals. Fifty six percent (56%) of them harbored one strain of Mutans Streptococci, while 40% had more than one strain and 4% were negative for MS on mitis salivarius agar. The corresponding mean DMFT/dmft scores were 4,5 respectively, and the average decayed teeth was 2.8,3.6 (figure 4).

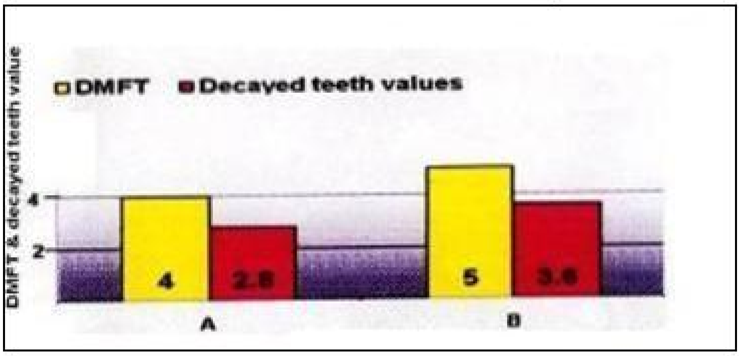

Figure 4: DMFT/dmft and decayed teeth component value in:
A: samples harboring one strain of MS.
B: samples harboring more than one strain of MS.

These results indicate that subjects harboring more than one strain have a higher incidence of dental caries than those positive for *S. mutans* alone. Similar results were reported by (16).

S. mutans is a potent initiator of caries because there is a variety of virulence factors unique to the bacteria that have been isolated that play an important role in caries formation. Firstly It is an anaerobic bacterium known to produce lactic acid as a part of its metabolism. Secondly, its ability to bind to teeth surfaces in the presence of sucrose by the formation of water-insoluble glucans, a polysaccharide that aids in binding the bacterium to the tooth. Mutant strains developed to produce water-soluble glucans instead have extremely diminished cariogenicity, especially on the smooth surfaces of the teeth which requires greater tenacity for binding to occur. Water-insoluble glucan has been found to lower the calcium and phosphate concentration of saliva, decreasing its ability to repair the tooth decay caused by bacterial lactic acid (17). The most important virulence factor, however is the acidophilicity of the bacteria. unlike the majority of oral microorganisms, *S. mutans* thrives under acidic conditions and becomes the dominant bacterium in cultures with permanently reduced PH.

The primary virulence traits of *Streptococcus mutans* are sucrose-dependent adherence, aciduricity and acidogenicity (11). Sucrose-dependent adherence is mediated by glucans, the products of the enzymatic glucosyltransferases (GTFs) (12) and it is initial step in the formation of biofilm communities. Eight TTC positive isolates (figure 4) which gave dark pink colonies when sprayed with 2,3,5-triphenyltetrazolium chloride (TTC), identified as *Streptococcus mutans* demonstrated various degrees of adherence in 2% sucrose medium (figure 5).

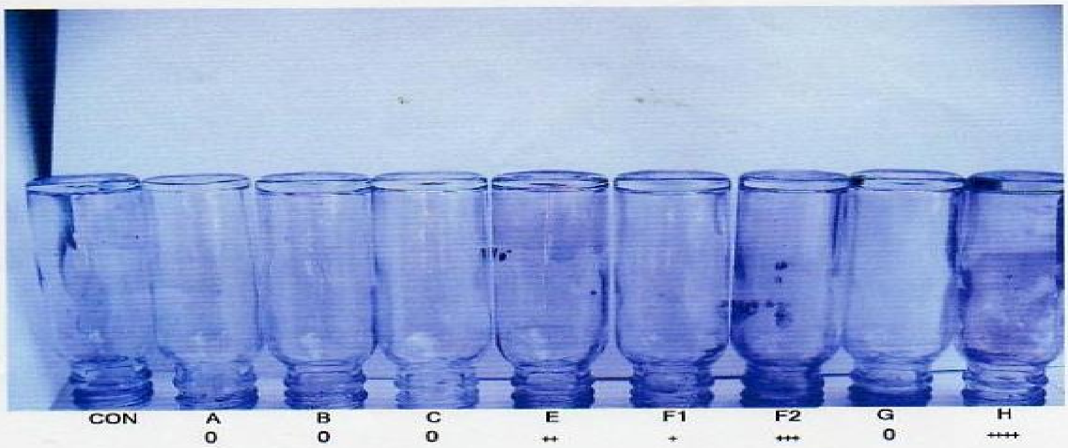

fig 5 : *Adherance of Streptococcus mutans grown in TSB + 2% sucrose.*

Colonies of strains F2 and H on MSA were surrounded by a mucoid layer about twice the size of the colony. Visual inspection of the tubes showed various degrees of adherence to the sides and bottom of tubes degree of adherence might be due to the production of high level of water-insoluble glucan (WIG) . further analysis of the exudates is needed to confirm this presumption. On the other hand, no adherence was observed in tubes A,B,C and G. studies have shown that two classes of enzymes, one which produces WSG and one which produces water-insoluble glucan (WIG) can be identified (19). When both enzymes are present, wild type *S. mutans* produces mostly adherent WIG, whereas only small amount of the non-adherent WSG can be detected (19).

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