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RELATIONSHIP OF GLUCAN SYNTHESIS AND
BACTERIOCIN ACTIVITIES TO MUTANS STREPTOCOCCI
TRANSMISSION

by

Joanne Ho Yan Leung

THESIS

submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Oral and Craniofacial Sciences

Abstract

Objectives: The aims of the study were to investigate whether: 1) mutacin activity of MS against other oral streptococci; 2) sensitivity of mutans streptococci (MS) to bacteriocin produced by other closely related oral streptococci, namely *S. sanguinis* and *S. gordonii*; 3) and formation of extracellular insoluble glucans by MS are associated with MS transmission from mothers to their children.

Methods: Sixteen transmitted MS genotypes and 27 non-transmitted MS genotypes from 13 mothers who had transmitted MS to their children were included in the study. The sensitivities of MS to bacteriocin produced by *S. sanguinis* 10556 and *S. gordonii* 10558 were evaluated by bacterial overlaying assay. Mutacin activities of transmitted and non-transmitted MS against *S. sanguinis* 10556*, S. gordonii* 10558, *S. mutans* 25175, and *S. sobrinus* 6715 were also analyzed by similar assay. Anthrone-sulfuric acid colorimetric assay was used to quantify the extracted extracellular polysaccharide produced by transmitted and non-transmitted MS.

Results: Mutacin activities of transmitted MS genotypes against *S. mutans* and *S. sobrinus* were significantly higher than those of non-transmitted MS genotypes (Student t test, *P<.05*). There were no significant differences in mutacin activity against *S. sanguinis* and *S. gordonii*, nor on the sensitivity to bacteriocin produced by *S. sanguinis* and *S. gordonii* between transmitted or non-transmitted MS (Student t test, *P>.05*). The amount of glucan produced was not statistically related to MS transmission (Student t test, *P<.05*).

Conclusions: Higher mutacin activities against other *S. mutans* and *S. sobrinus* in maternally-transmitted MS genotypes may be an important contributing factor for their oral colonization in children, as compared to non-transmitted maternal strain. MS mutacin activities against *S. sanguinis* and *S. gordonii*, MS sensitivities to bacteriocin(s) produced by *S. sanguinis* and *S. gordonii* and MS glucans synthesis appear may not be related to transmission of MS in children.

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1. Introduction

Dental caries is the most prevalent infectious disease in children.[1] Mutans streptococci (MS), namely *S. mutans* and *S. sobrinus,* have been recognized as the major cariogenic bacteria in children with early childhood caries.[2-4] Research has shown early acquisition of MS as a main risk factor for early childhood caries and future caries experience in primary and permanent dentition. Four-year-old children who had detectable MS at age 2 had significantly higher caries prevalence than children with later colonization or non-colonization[6, 7]. MS infection initiates with successful spread of bacteria and subsequent colonization on newly erupted teeth.[7] Change of dietary habits including increased consumption of sucrose increases the risk of MS transmission.[2] In addition, young children are facing the challenge of cariogenic microorganism before "maturation" of enamel surface. Colonization of MS can be observed in infants as early as 3 months of age in infants.[8] The colonization rate of MS in children increases with age and as more teeth erupted in oral cavity. By understanding the mechanism of acquisition of MS in infants, we can develop a regimen to prevent or delay MS colonization in infants and thereby prevent early childhood caries.

Transmission of MS in children can come from many different sources. Vertical transmission refers to the transmission of microbes from caregiver to child.[5] Several studies identified maternal transmission as a major route of vertical transmission for MS.[6, 9, 10, 35] Other studies also demonstrated transmission of MS to children from fathers.[11] Horizontal transmission is defined as the transmission of microbes between members of a group (e.g., family members of a similar age or students in a classroom etc.).[5, 12, 13] Homology of MS genotypes between spouses and homology of MS genotypes

in children in the same nursery school sharing same genotypes are examples of horizontal transmission.[11, 14, 15]

It is interesting to note that not all MS genotypes present in from the sources were transmitted to infants or children in either vertical or horizontal transmission studies. In Kohler's study, only 67% of recovered maternal genotypes were shared by their children.[6] Furthermore, Caufield's study showed that only about 50% of maternal genotypes were transmitted to their infants.[10] These findings regarding MS transmission have led us to hypothesize that successfully transmitted maternal MS genotypes may have specific characteristics that enhance their transmission. However, very few studies have investigated possible virulence factors that are related to transmission of MS.

We hypothesize that three virulence factors may relate to MS transmission, including: 1)mutacin formation; 2) sensitivity to bacteriocin formed by other common oral bacterial; and 3)extra-cellular polysacharride formation ability.

2. Literature Review

2.1 Mutacin Production by Mutans Streptococci

Mutacin, a group of bacteriocins produced by MS, are peptides that can be bactericidal to closely related oral streptococci.[16, 17] It helps MS to fight in the micro-niche against oral microbes for successful colonization. Mutacin may play an important role for equilibrium of dental plaque composition. Very few studies have investigated the relationship between mutacin production by MS and its transmission potential. Gronorrs et al investigated the relationship of transmission and mutacin production of MS by comparing mutacin activity of transmitted and non-transmitted MS isolated from 13 mother-child pairs against 14 other oral streptococci strains.[9] Maternal transmission rate

for MS to their 18-month to 3-year-old children based on ribotyping and same mutacin typing results was 64%. They also found that the transmitted MS has a significantly broader mutacin activity and bigger inhibition zone against eight of the 14 indicator strains (*S. mutans* MT 8148 (serotype *c*), *S. mutans* OMZ175 (*f*), *Streptococcus rattus* FA-1 (*b*), *Streptococcus sanguis* ST202, and B220, *Streptococcus gordonii* ATCC 10558, *Streptococcus salivarius* HHT, and *Streptococcus pyogenes* SV) compared to the non-transmitted ones which implies that the transmitted MS had higher mutacin activity than the non-transmitted ones. Their study also indicated the mutacin activity of MS stayed consistent in most of 10 mothers and their 5-year-old children at a 1-7 year interval. They therefore suggested that mutacin may be a significant virulent factor in dental caries.[9] Additionally, Hillman had found a relationship between mutacin and *S. mutans* ability to persistently colonize in the oral cavity and to replace the originally colonized mutans streptococci.[40] Study by Li et al showed that MS strains carried mutAI gene were identified as non-transmitted strains suggesting the presence of mutAI gene may related to MS transmission.[16] Therefore, mutacin formation may serve as a potential virulence indicator for MS transmission.[7, 9] However, there is very limited research data to confirm this hypothesis in children who developed ECC.

2.2 Sensitivity to Bacteriocin produced by other close related oral streptococci

The second virulence factor that may relate to MS transmission is the sensitivity of MS to bacteriocin(s) produced by other dominant bacteria in dental plaque, such as *S. sanguinis* and *S. gordonii*. *S. sanguinis* and *S. gordonii* are earlier and more predominant colonizers in dental plaque of sound tooth surface than MS.[19, 20] They are both

considered as the health-associated microrganisms which compete with oral pathogens, such as MS, to maintain oral health. Studies have demonstrated that *S. sanguinis* is associated with healthy tooth surfaces but not with caries.[20-22] Moreover, early colonization with *S. sanguinis* was significantly correlated with delayed colonization with *S. mutans* in children.[19] Also, caries-free children had higher relative level of *S. sanguinis* in relationship to MS levels than children with severe early childhood caries (S-ECC).[23] It was hypothesized that there is an antagonistic colonization correlation between MS and *S. sanguinis* in the oral cavity. Similar to MS, both *S. sanguinis* and *S. gordonii* produce bacteriocins to inhibit other close related bacterial growth. We are hypothesizing that the ability of MS to overcome the suppression factors from the early colonizers may determine whether it could successfully colonize in infants. However, no study has investigated potential relation of sensitivity of MS to bacteriocin of other oral streptococci to its ability of transmission.

2.3 Glucan Synthesis by Mutans Streptococci

Third, glucan formation may serve as another possible virulent factor associated with MS transmission. Glucan is synthesized from sucrose by the enzymatic action of glucosyltransferases (GTFs) in MS. Glucan, especially water-insoluble glucan, plays an important role in attachment of plaque and MS.[44] Glucan formed by GTF facilitate MS adhesion to plaque due to its physical and chemical properties. *S. mutans* have GTF on their surfaces and release GTF molecules to the environment where it can adsorb to solid tooth surfaces and to other microorganisms.   Glucans are then produced by the absorbed GTF molecules. There are 3 types of absorbed GTF molecules: GTFB and GTFC synthesize mainly water-insoluble glucans (>85%) with $\alpha(1-3)$ glucosidic bonds (mutan);

GtfD forms water-soluble glucans (>70%) with α(1–6) glucosidic bonds (dextran)[29]. The α(1-3)-rich insoluble glucan is associated with more tenacious adhesion to the tooth surface, with dental plaque development, and with smooth surface decay formation through several mechanisms.[29]   On the other hand, extrinsic dextran competitively reduces the adherence of microorganisms to glucan-coated apatitic surface.[39]

GTF presents in all layers of pellicle and can produce glucan *in situ*[26]. MS can then bind to glucan synthesized on tooth surface via its well-characterized surface proteins, glucan-binding proteins (GBPs).[30] *S. mutans* secrete GTFs which bind avidly to the pellicle formed on the tooth surface and to bacterial surfaces and are enzymatically active.[38] Glucans can be formed *in situ* within minutes after exposing to sucrose. The glucans provides binding site for colonization and accumulation of *S. mutans* on the apatite surface and for binding to each other through interactions with several membrane-associated GBPs and surface glucans. Furthermore, Mattos-Graner has shown a positive relationship between water-insoluble glucan and caries incidence, implying that various levels of GTF activity can result in different virulence of *S. mutans*.[47] The mean amount of water-insoluble glucans, which is synthesized by GTF, is shown to be less in caries-free subjects even though there is same amount of MS colonization as caries-active subjects. This means water-insoluble glucan may play a very important part in making the dental plaque more cariogenic and play a role in caries transmission. However, this study only included MS isolates from very limited numbers of subjects.

3. Preliminary data:

Previously, Drs. Tan and Zhan have conducted a study to investigate whether transmission of MS is associated with certain virulence factors, such as biofilm formation

and mutacin formation.[46] In that study, 10 mother-child pairs with high-caries risk were recruited for the study. All mothers were caries-active and primary care givers of their children aged 2-6 years old. Ten MS colonies were isolated from each subject and genotyped by AP-PCR with two primers (OPA 05 and OPA 13) . All mothers and their children had MS colonization. The study identified 36 MS genotypes from the mother but only 40% of children had mother's MS genotype and only 7 maternal MS genotypes were transmitted to their children. No statistically significant difference in biofilm formation between transmitted and non-transmitted MS genotypes was noted in the study. Although there was a consistent trend on stronger mutacin formation by transmitted MS genotypes against *S. sanguinis* and *S. gordonii*, no statistically significant difference was noted between transmitted and non-transmitted genotypes which is different from the result of Gronroos' study[9]. In Tan's study, transmitted MS genotypes produced significantly more mutacin against *s. sobrinus* than non-transmitted genotypes. The results of the study indicated mutacin formation by MS may be related to MS transmission.   There are a few limitation of the study: first, the low maternal transmission rate of MS limited the numbers of MS genotypes in the study. Second, the study included MS from 6 mothers who did not have MS transmission to their children. This might introduce the bias for the study because behavior factors other than MS virulence may interfere the MS transmission. Third, MS biofilm formation can be potentiated by bacteria growth and other factors. This cannot accurately represent the direct formation of glucans formation. Therefore further studies should include a bigger sample size and more precise assays on glucan formation.

4. Study Hypothesis and Aims

The hypothesis of the study is that: 1) transmitted MS genotypes will produce more mutacin and glucans than non-transmitted MS genotypes; 2) transmitted MS genotypes will be less sensitive to bacteriocin produced by other oral streptococci in dental plaque, such as *S. sanguinis* and *S. gordonii*. The aims of the study are to investigate whether maternal MS transmission to child is related to : 1) MS mutacin activity against other oral Streptococci; 2) sensitivity of MS to bacteriocin produced by other oral streptococci, namely *S. sanguinis* and *S. gordonii*.; and 3)extracellular insoluble glucans formation in a big population. The study population should only include mother-child pairs who had maternal MS transmission to children.

5. Materials and Methods

5.1 Study Population:

MS genotypes isolated from mothers who had maternal MS transmission to their children from two populations in two previous studies were included in the current study. In both studies, a dental exam and stimulated saliva samples from mothers and swab saliva samples from children were collected and cultured anaerobically for 72 hours before enumeration. Ten MS colonies were isolated from each of the subjects, genotyped by AP-PCR and phenotyped by fermentation test as described previously. [45, 46]

These included 4 mother-child pairs recruited for Dr. Ten's study. Eighteen MS isolates in Ten's study were used in the current study (7 transmitted MS genotypes and 11 non-transmitted MS genotypes). The other population included mothers with active caries who had young children aged 6 months to 3 years old. Nine mother-child pairs were included in this study. All mothers in both groups had maternal transmission of MS to their children. Twenty-five maternal genotypes were isolated in the 2nd population

with 9 transmitted and 16 non-transmitted MS genotypes. The demographic data of the study population are summarized in Table 1.

Pt's ID	Gender	Age (months)	dmfs – child	DMFS - mother
1	F	13	0	19
2	F	34	0	68
3	M	31	0	4
4	M	29	0	3
5	M	22	22	6
6	M	28	0	5
7	M	20	0	9
8	F	9	0	2
9	M	48	3	14
10	M	24	3	21
11	F	60	1	48
12	F	48	0	19
13	F	24	48	17
Mean (SD)	n/a	30	5.9	18.1

Table 1. Demographic Data of Study Population

5.2 Inclusion Criteria

- Children aged 6 months to 4 years old

- All children were at high caries risk

- All mothers had active caries within 1 year

- Mothers who are the primary care givers of their children

- Mother had at least one MS genotypes transmitted to the child.

5.3 Exclusion criteria:

- Mother or child with systemic disease or periodontal disease

- Antibiotic or medication use within the past three months that may alter oral flora.

5.4 Mutacin Activity Assays

The stored MS genotypes in Tryptic Soy Broth (TSB)-20% glycerol broth at -80°C was grown in brain-heart infusion broth (BHI) at 37°C for 24 hrs. Then they were

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inoculated by stabbing 4 times with a 27 gauge needle into TSB plates containing 1% (w/v) agar and supplemented with 0.5% (w/v) yeast extract and incubated for 48 hours.

S. mutans GS-5 was used as the positive control strain and was stabbed on the TSB agar plate as if it were for a test strains. Reference strains, *S. sanguinis* 10556*, S. gordonii* 10558, *S. sobrinus* 6715 and *S. mutans* 27175, were used as indicator strains. These reference strains were cultured in TSB for 16 hours, washed with phosphate-buffered saline (pH7,3) and re-suspended in the same buffer to an absorbance between 0.06 and 0.09 at 490nm. A 0.1 ml volume of each indicator strain suspension was then be inoculated into 4.5ml of semisolid trypticase soy agar (0.75% w/v agar and 0.5% w/v yeast extract) and overlaid on the stab plates.

The inhibition activity was evaluated after 48 hours of incubation at 37˚C in anaerobic condition (10% H_2, 10% CO_2, and 80% N_2). by measuring the radius of each inhibition zone with magnifying lens. The process was in duplicates and was repeated twice and the mean size of the inhibition zone was used for statistical analysis. The procedure of the mutacin assay is summarized in Figure 1.
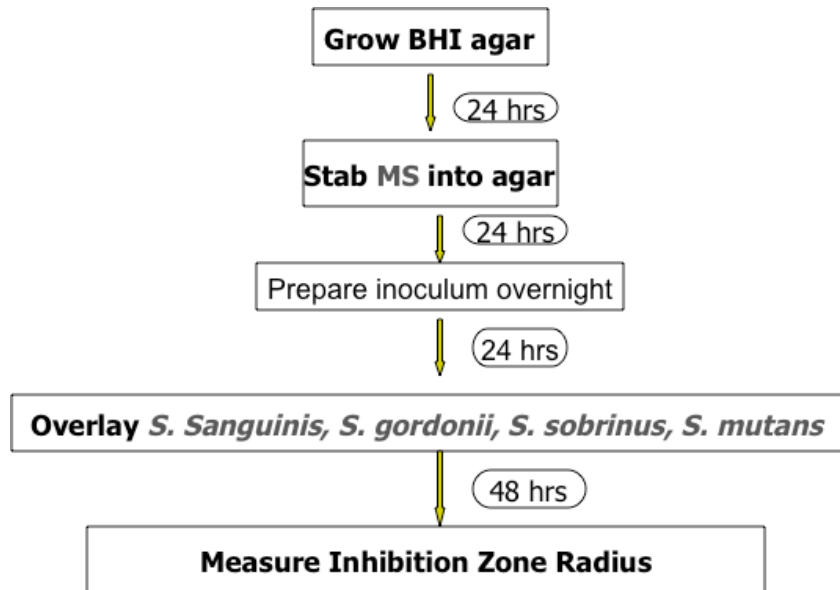
Figure 1 Flowchart of mutacin production assay

5.5 Sensitivity of MS to Bacteriocin Produced by *S. sanguinis* and *S. gordonii*

The assay was the same as mutacin assays except that *S. sanguinis* 100556 and *S. gordonii* 10558 reference strain were stabbed by needle on the TSB plate while the isolated transmitted and non-transmitted MS were inoculated in the overlay layer to observe inhibition of bacteriocin secreted by *S. sanguinis* 100556 and *S. gordonii* 10558 against the growth of overlaid MS isolates. Procedures of this assay is summarized in Figure 2.

Grow BHI agar

↓ (24 hrs)

Stab *S. sanguinis* and *S. gordonii* into agar

↓ (24 hrs)

Prepare inoculum overnight

↓ (24 hrs)

Overlay MS

↓ (48 hrs)

Measure Inhibition Zone Radius
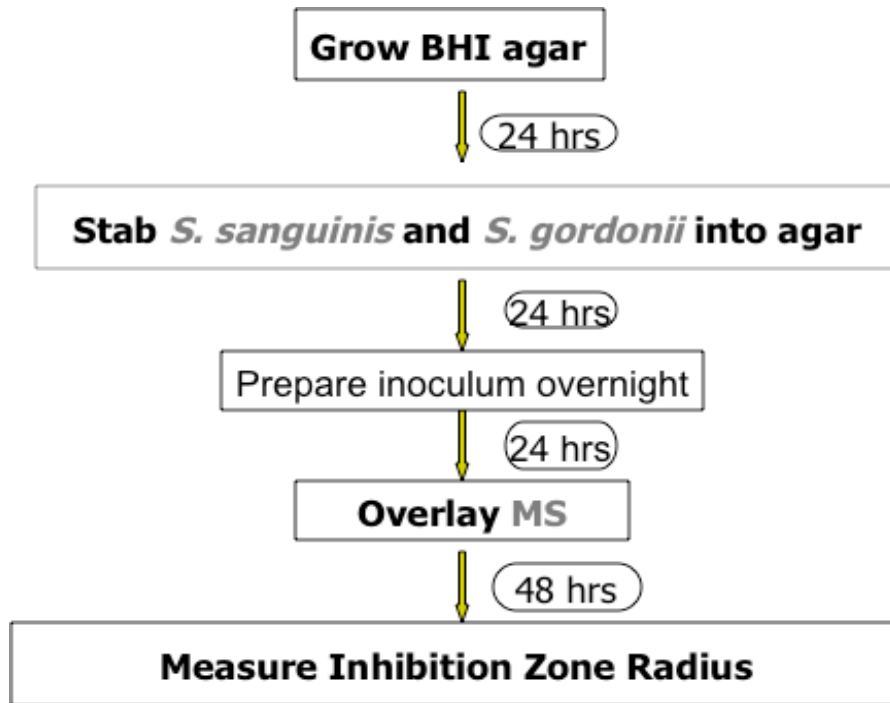
**Repeat process twice

Figure 2. Flowchart of bacteriocin sensitivity assay

5.6 Water-insoluble Glucan Synthesis

MS strains were recovered from frozen stock on BHI plate and then were inoculated in 2ml of TPY broth for overnight culture. Then, 0.1ml of bacterial overnight culture was inoculated into 2ml TPY broth with 5% sucrose and incubated anaerobically for 48 hours. This broth was centrifuged at 10000 RPM to collect cell pellet was washed 2 times with 2ml PBS.

Two ml of 0.5N NaOH was added to the pellet and incubated in 60°C water bath for 10 minutes to extract the water-insoluble glucan. The suspension was centrifuged and supernatant was collected into a 50ml centrifuge tube. This step was repeated twice and the total amount of 6 ml supernatant was collected together for water insoluble glucan extraction.

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Polysaccharide was then extracted and by adding and thoroughly mixing 3 volumes of pure ethanol to the tube of collected supernatant solution and allowed to precipitate overnight. This precipitate was collected by centrifuge and dried. It was then dissolved in 0.5N NaOH for water insoluble polysaccharide.

Anthrone-sulfuric acid colorimetric assay was used to quantify the extracted extracellular polysaccharide. Five Standards (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, and 0.4g/L glucose) and control were prepared. The extracted polysaccharide was added with 2mL of 0.5N NaOH and then incubated in 60ºC water bath for 10 minutes Four hundred ul of anthrone 0.012M +75% sulfuric acid reagent were mixed with 100 ul of sample. Samples then were incubated in water bath at 100ºC for 12 minutes. Samples were cooled to room temperature. After that, 100 ul of the sample was pipeted into a 96 well micro plate and the absorbance at 650nm was measured using a Molecular Devices microplate spectrophotometer (Sunnyvale, CA). *S. sobrinus 6715* was used as the positive control strain. Polysaccharide concentrations were determined from an established glucose standard curve.

5.7 Statistical Analysis

Diameter of inhibition zone from bacteriocin sensitivity assay, means and standard deviations of milligrams per ml water-insoluble glucan formed in the glucan assay were calculated for both transmitted and non-transmitted MS groups. The values of these assays will also be compared by independent Student t tests at alpha =.05 to see whether these factors are related to the transmission of MS.

6. Results

6.1 Mutacin Acitivity

Mutacin activities of transmitted MS genotypes against *S. mutans* 25175 and *S. sobrinus* 6715 were statistically significantly higher than those of non-transmitted MS genotypes (Figure 3, Student t-test, *P<.05)*. There was no significant difference in mutacin activities against *S. sanguinis* 10556 and *S. gordonii* 10558 between transmitted or non-transmitted MS *(*Figure 3, Student t-test, *P>.05)*
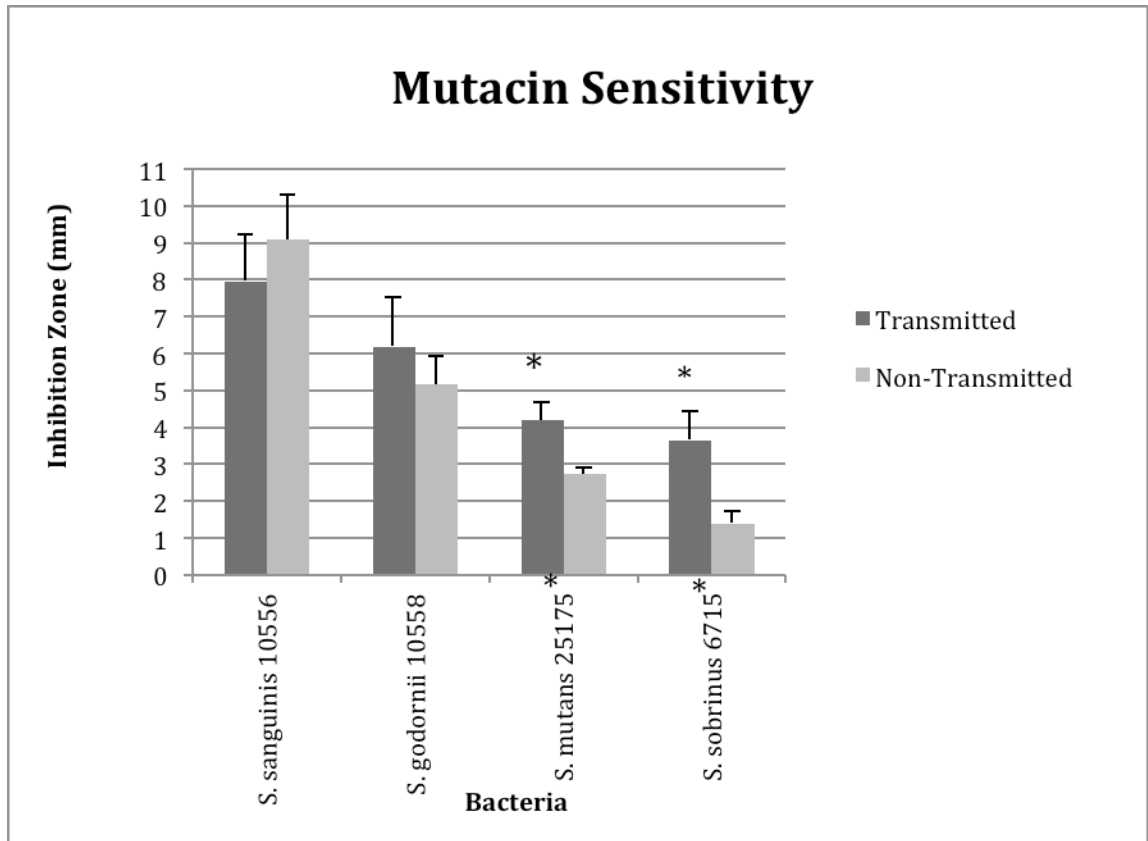
Figure 3. Sensitivity of indicator strains to mutacin produced by transmitted & non-transmitted MS

6.2 Bacteriocin Sensitivity

There was no statistical significant difference in the sensitivity to bacteriocin produced by *S. sanguinis* 10556 and *S. gordonii* 10557 between transmitted and non-transmitted MS *(*Figure 4, Student t-test, *P>.05.)*

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Bacteriocin Sensitivity
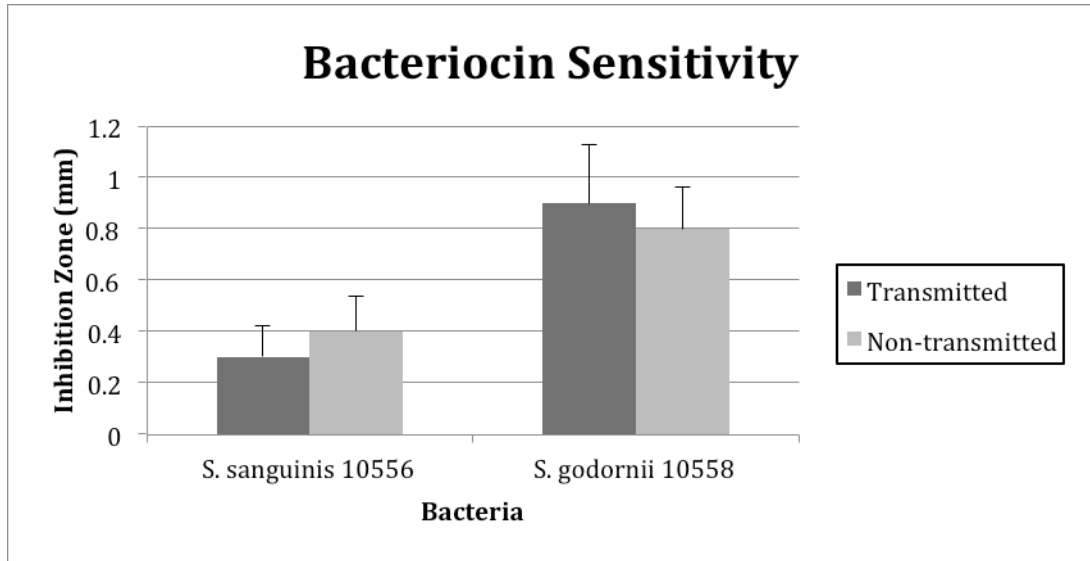
Fig. 4 Sensitivity of transmitted & non-transmitted MS strains to bacteriocins produced by *S. sanguinis* & *S. gordonii*.

6.3 Insoluble-Glucan Assay

There was no statistical significant differences in glucan production between transmitted and nontransmitted MS genotypes (Figure 5).
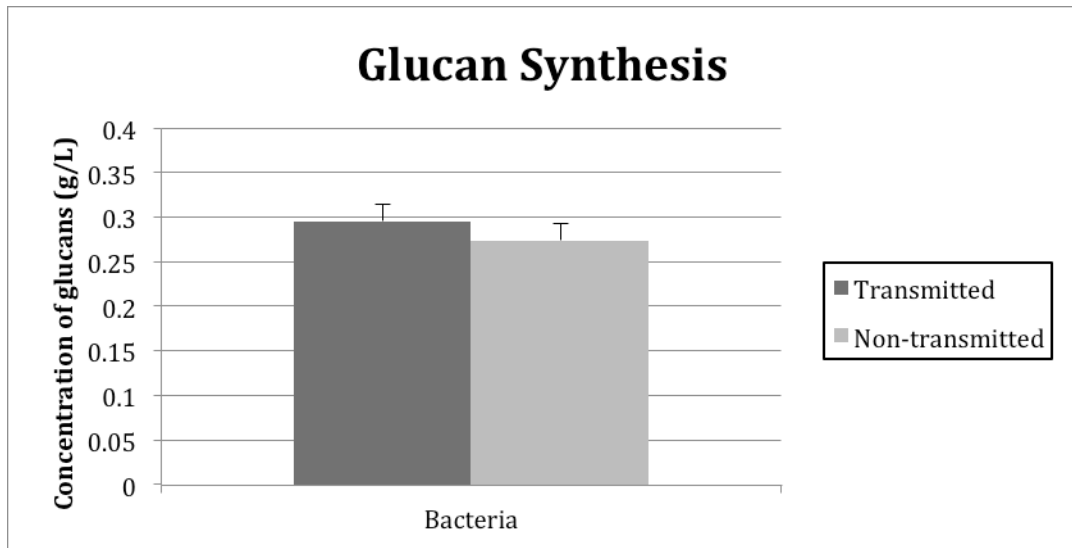
Glucan Synthesis

Figure 5 Glucan synthesis by transmitted & non-transmitted MS strains.

7. Discussion

Since dental caries is one of the most common infectious diseases in children and early colonization of MS is correlated to high risk of ECC[1], it is important to explore ways to prevent caries transmission. The current study focused on whether 3 virulence factors: mutacin formation, bacteriocin resistance and extracellular insoluble glucans formation are related to transmission of MS, the major cariogenic bacteria. Our results indicate that transmitted MS has higher mutacin activities against other *S. mutans* and *S. sobrinus*. These results are in agreement with a previous small-sample study by Tan et al which indicated that mutacin activity against *S. sobrinus* may be significantly related to transmission. Also, similar to the Tan pilot study, we did not find a significant higher mutacin activity from transmitted MS against *S. sanguinis* and *S. gordornii* compared to non-transmitted MS. Mutacin's role in MS transmission is unclear *in vivo*. However, it's suggested that it provides an ecological advantage to producing strains in the bacterial community and supports the establishment of *S. mutans in vivo*.[37] Several studies have implied that bacteriocin(s) of mutans streptococci (mutacins) can help promote MS transmission from one individual to another. Some have suggested that higher mutacin production facilitate MS colonization and increases transmission opportunities and to young children [9, 41, 42, 43] Rogers reported that produced bacteriocin could aid in *S. mutans* colonization. In the current study, with a larger sample size, we have confirmed a correlation between mutacin formation against *S. mutans* 27175 and *S. sobrinus* 6715 and MS transmission. However, no significant difference was noticed regarding mutacin formation against *S. sanguinis* and *S. gordonii*. The result appears surprising that the transmitted MS genotypes that can be transmitted are the ones that have a strong inhibitory effect against other MS strains, but not to other close-related oral streptococci.

This implies that strong mutacin production provides a transmission advantage to specific MS strains.

Because MS is a later colonizer among the oral streptococci, we were also interested whether tolerance to bacteriocin(s) produced by other established oral streptococci affect MS colonization or transmission. Caufield et al. observed that early colonization of *S. sanguinis* in infants was significantly related to delayed MS colonization.[19] It has also been suggested that *S. sanguinis*-serves a protective role against *S. mutans*.[23] In addition, *S. sanguinis* is higher in number in caries-free children than in severe early childhood caries children. However, in the current study, we did not find statistically differences in sensitivity to bacteriocin activities between the transmitted and non-transmitted MS genotypes. This suggests bacteriocins from *S. sanguinis* or *S. gordornii* do not affect transmission of MS. However, this conclusion needs to be drawn with caution because the current study only included two reference strains and the wild types of oral streptococci vary at individual level. Further studies will be needed to identify interactions between MS and other streptococci that are isolated from the same subjects.

Glucan synthesis was also seen as one potential of virulence factors related to MS transmission. GTFs can be present as a part of dental pellicle.[26] They are released by both *S. mutans* and *S. sanguinis*. GTFB has been shown to significantly correlate with dental caries.[49] In addition, Munro suggested that both GTFB and GTFC play a role in smooth surface caries in a rat model.[50, 52] Using atomic force microscopy, Cross has demonstrated that glucans aid with *S. mutans* adherence and its strength increases over time.[48, 51] Through production of glucans with different function and structure, *S. mutans*

GTF contributes to virulent plaque production and their products play important roles in dental caries pathogenesis. However, we did not find a statistically significant relationship between glucans formation and MS transmission. Based on our study result, the quantity of glucans is not related to MS transmission; however, this study only explores the amount of synthesis in relationship with transmission but did not investigate other properties, such as structure or expression, of glucans and their relationship with plaque formation and MS adhesion. Further research should be carried out to further look into these properties.

There are certain limitations in the study. First, though the mother is the primary caregiver of the children, the children may have acquired MS from other sources such as through siblings, playmates and other caregivers. If this factor is taken into account, the result of the study may be different. There may be competitions among MS that is transferred from mother as a source and MS that is transferred from non-mother sources. This will affect the final amount of MS that can be transmitted from mother to their children. Secondly, since different GTFs can produce different glucans with different function, the result may be more meaningful if glucan is further categorized based on types before determining its association with dental caries. In the future, one could explore to other more possible virulence factors of MS transmission and how they relate to transmission. After studying each factor thoroughly, then one can combine all the factors and create an overall model that explains this transmission. Third, only limited reference strains from ATCC were used as indicator strains for MS mutacin activity or as primary inoculate to test MS resistance to bacteriocin. These limited strains may not represent the variety of genotypes that are present in children's oral flora. Future studies

should include more variety of reference or indicator strains or indigenous bacterial isolates from children and/or mothers to study these interactions. Therefore, we should draw the conclusions from this study with caution.

8. Conclusions

This study has shown that there may be a significant correlation between mutacin activities of transmitted MS against other *S. mutans* and *S. sobrinus*. On the other hand, we did not find significant correlation between sensitivity of transmitted MS to bacteriocin formed by *S. sanguinis* and *S. gordonii,* or extracellular water-insoluble glucan synthesis to MS transmission. MS transmission is a complex process. Other virulent factors may also affect the transmission of MS. We have just explored three of these factors. Further research should be carried out to explore other virulence factors. It is very labor intensive to study virulence factors by traditional biological methods. Therefore, future study may involve newly emerging genetic sequencing or gene expression assays that allow high-throughput screening for presence or absence of panels of genes in bacteria to facilitate quick identification of candidate virulence factor or genes for further investigation. It is important to recognize these factors in order to create an effective dental caries preventive program.

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10. Appendices

Appendix I: Diameter of Inhibition Zone by transmitted and non-transmitted MS mutacin

Inhibition Zone(mm) – Mutacin by transmitted MS

Strain ID	S. sanguinis 10556 mean	SD	S. gordonii 10558 mean	SD	S. mutans 25175 mean	SD	S. sobrinus 6715 mean	SD
S1M-4	12	1.3	11.2	3.7	2.2	0.2	6.5	1.3
S4M-10	6.12	1.61	8.8	0.4	2.0	0.4	6.9	1.7
S5M-1	3.75	0.37	2.6	0.9	1.8	0.4	0.0	0.0
S9M-10	4.39	0.57	2.7	0.2	2.9	0.4	8.7	1.4
S9M-2	3.61	0.84	2.4	1.6	3.8	0.9	6.1	0.7
S9M-4	3.78	0.69	2.6	0.6	4.1	0.4	6.7	2.0
S9M-8	4.27	1.37	3.0	0.2	2.7	0.7	7.7	0.7
SW06-8	12.7	2.25	20.9	2.4	8.8	1.3	0.0	0.0
SW06-9	8.15	3.24	8.9	1.7	2.9	0.3	0.0	0.0
SW07-2	11.1	0.48	13.8	2.3	5.0	0.8	4.1	1.0
SW17-3	3.16	1.52	2.1	0.7	2.7	0.7	1.2	1.4
SW19-4	11.2	1.84	3.9	0.4	4.3	0.6	2.7	0.8
SW28-2	4.18	0.67	2.7	1.2	3.2	0.7	0.0	0.0
SW30-9	21.4	3.97	2.8	0.2	2.6	0.3	1.5	1.6
SW33-2	13.6	0.95	4.5	0.4	4.4	0.5	2.5	0.4
SW42-2	3.94	0.44	6.1	0.6	3.8	0.4	3.8	1.3

Inhibition Zone (mm) – Mutacin by non-transmitted MS

Strain ID	S. sanguinis 10556 mean	SD	S. gordonii 10558 mean	SD	S. mutans 25175 mean	SD	S sobrinus 6715 mean	SD
S1M-1	7.8	2.2	3.6	1.0	2.4	0.5	0.0	0
S1M-3	9.0	1.3	3.6	0.8	2.2	0.4	0.0	0
S1M-6	9.2	0.9	3.0	0.4	1.9	0.3	0.0	0
S4M-1	3.9	0.7	3.9	1.3	1.9	0.7	0.0	0
S4M-4	3.8	1.4	3.5	0.4	1.5	1.3	0.0	0
S4M-5	2.2	0.7	3.5	0.6	1.0	1.3	0.0	0
S5M-4	8.6	2.0	3.6	0.9	3.5	0.4	5.3	1.2
S5M-6	2.0	0.1	4.5	1.2	2.0	0.5	2.1	0.3
S9M-1	3.3	1.0	0.0	0.0	2.5	0.6	0.0	0
S10M-1	2.8	0.3	3.4	1.8	2.1	0.3	0.0	0
S10M-4	2.3	0.5	3.7	0.7	2.4	0.3	0.0	0
SW06-1	21.8	1.7	15.2	1.4	2.0	2.2	0.0	0
SW06-6	9.5	1.7	3.1	1.6	2.2	2.4	0.0	0
SW07-1	10.9	0.8	13.9	2.1	4.9	0.8	3.8	0.6
SW07-10	10.5	1.8	11.6	3.8	2.8	3.1	1.9	2.5
SW07-7	9.5	0.5	3.1	1.2	2.8	0.5	0.8	0.9

SW17-2	2.2	0.6	2.2	0.8	4.0	0.4	0.0	0
SW19-2	22.2	4.0	14.2	2.7	3.7	1.2	1.4	1.5
SW19-9	6.1	0.8	6.7	0.6	4.3	1.0	1.9	2.1
SW28-3	5.0	1.5	2.7	0.3	3.1	0.5	2.5	0.4
SW28-5	12.1	0.9	5.1	0.9	3.1	0.5	1.8	1.9
SW30-1	11.4	1.4	8.3	0.6	1.8	2.0	5.5	0.5
SW30-2	8.6	1.3	1.6	1.3	3.1	0.9	0.0	0
SW30-4	10.3	1.5	7.5	1.3	3.5	1.4	4.8	1
SW30-5	5.7	0.7	2.9	0.7	3.7	0.7	2.2	1.3
SW30-7	22.5	2.5	2.9	0.2	2.0	1.8	1.5	1.7
SW30-8	22.1	2.8	2.2	1.4	3.2	1.0	2.5	0.3

Student t-test result

Strains	P value	Statistically significant?
S. sanguinis 10556	0.479	No
S. gordonii 10558	0.484	No
S. mutans 25175	0.036	Yes
S. sobrinus 6715	0.004	Yes

Appendix II: Diameter of Inhibition Zone by Bacteriocin

Inhibition Zone (mm) – Bacteriocin against transmitted MS

Strain ID	S. sanguinis 10556 mean	SD	S. gordonii 10558 mean	SD
S1M-4	0.0	0.0	0.6	0.7
S4M-10	0.0	0.0	1.5	1.6
S5M-1	0.8	0.9	2.2	0.4
S9M-10	0.0	0.0	0.0	0.0
S9M-2	0.0	0.0	0.5	0.6
S9M-4	0.0	0.0	0.4	0.4
S9M-8	0.0	0.0	0.4	0.4
SW06-8	0.8	1.8	0.4	0.4
SW06-9	0.0	0.0	2.9	1.5
SW07-2	0.0	0.0	0.0	0.0
SW17-3	1.6	1.0	2.2	1.4
SW19-4	0.6	0.7	1.3	0.3
SW28-2	0.0	0.0	0.0	0.0
SW30-9	0.0	0.0	1.1	1.6
SW33-2	1.0	1.1	1.7	0.9
SW42-2	0.0	0.0	0.0	0.0

Inhibition Zone (mm) – Bacteriocin against non-transmitted MS

Strain ID	S. sanguinis 10556 mean	SD	S. gordonii 10558 mean	SD
S1M-1	2.1	0.3	2.4	0.4
S1M-3	0.0	0.0	1.4	1.5
S1M-6	2.4	0.3	1.7	1.8
S4M-1	0.7	0.8	2.1	0.4
S4M-4	0.5	0.8	2.0	0.6
S4M-5	1.3	0.8	0.7	0.8
S5M-4	0.9	1.3	1.4	0.9
S5M-6	0.7	0.7	1.4	0.3
S9M-1	0	0	0.0	0.0
S10M-1	0	0	0.0	0.0
S10M-4	0	0	0.5	0.6
SW06-1	0.0	0.0	2.8	1.1
SW06-6	0.0	0.0	0.0	0.0
SW07-1	0.0	0.0	1.3	1.5
SW07-10	0.0	0.0	0.0	0.0
SW07-7	2.0	2.2	0.7	0.8
SW17-2	0.0	0.0	0.6	0.7
SW19-2	0.0	0.0	1.5	0.5

```
SW19-9        0.0   0.0        0.5   0.5
SW28-3        0.0   0.0        0.0   0.0
SW28-5        0.0   0.0        0.0   0.0
SW30-1        0.0   0.0        0.0   0.0
SW30-2        0.0   0.0        0.8   0.8
SW30-4        0.0   0.0        0.0   0.0
SW30-5        0.0   0.0        0.0   0.0
SW30-7        0.0   0.0        0.0   0.0
SW30-8        0.0   0.0        0.0   0.0
```

Student t-test result

Strains ID	P value	Statistically significance
S. sanguinis 10556	0.6	No
S. gordonii 10558	0.62	No

Appendix III: Glucan Synthesis

Glucan Synthesis Amount (g/L) - Transmitted MS

Strain ID	mean	SD	CV%
S1M-4	0.455	0.132	0.29
S4M-10	0.332	0.045	0.13
S5M-1	0.200	0.015	0.08
S9M-10	0.214	0.118	0.55
S9M-2	0.220	0.026	0.12
S9M-4	0.253	0.057	0.23
S9M-8	0.205	0.041	0.20
SW06-8	0.385	0.047	0.12
SW06-9	0.370	0.104	0.28
SW07-2	0.299	0.110	0.37
SW17-3	0.414	0.133	0.32
SW19-4	0.436	0.030	0.07
SW28-2	0.314	0.049	0.16
SW30-9	0.211	0.054	0.26
SW33-2	0.208	0.048	0.23
SW42-2	0.211	0.025	0.12

Glucan Synthesis Amount (g/L) - Non-transmitted MS

Strain ID	mean	SD	CV
S1M-1	0.251	0.088	35%
S1M-3	0.229	0.067	29%
S1M-6	0.416	0.061	15%
S1M-7	0.208	0.083	40%
S1M-8	0.233	0.063	27%
S4M-1	0.253	0.101	40%
S4M-4	0.498	0.187	38%
S4M-5	0.391	0.080	20%
S5M-4	0.208	0.071	34%
S5M-6	0.487	0.132	27%
S9M-1	0.056	0.032	57%
SW06-1	0.227	0.084	37%
SW06-6	0.166	0.070	43%
SW07-1	0.312	0.079	25%
SW07-10	0.272	0.027	10%
SW07-7	0.324	0.068	21%
SW17-2	0.144	0.063	44%
SW19-2	0.187	0.087	46%
SW19-9	0.395	0.168	43%
SW28-3	0.275	0.077	28%
SW28-5	0.410	0.098	24%

SW30-1	0.302	0.006	2%
SW30-2	0.113	0.026	23%
SW30-4	0.308	0.070	23%
SW30-5	0.259	0.097	38%
SW30-7	0.161	0.061	38%
SW30-8	0.283	0.039	14%

Student T-test: p value=0.49, not statistically significant

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