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Mutans Streptococci Enumeration and Genotype Selection using Different Bacitracin-containing Media

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Abstract

The primary etiological agents associated with dental caries include the mutans streptococci (MS) comprised of *Streptococcus mutans* and *Streptococcus sobrinus*. The effective cultivation and isolation of MS is necessary for the study of MS, including their proper clinical assessment in the epidemiological study of dental caries. Several selective media have been developed for the isolation, enumeration, and characterization of MS. However, inhibition of MS may occur, reducing counts and perhaps limiting selection of some strains. The purpose of this study was to compare five culture media containing bacitracin recommended for the isolation of MS.

Five commonly used bacitracin-containing media (MSB, MSKB, GTSB, TYS20B, and TYCSB) used for MS isolation were quantitatively evaluated. Standard plate counts were performed in

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The authors declare no conflicts of interest.

Supplementary materials

Additional supporting information may be found in the online version of this article:

Figure S1. Comparison of Colony Morphology for Prototype Strains on Five Media

Table S1. MS Prototype Mean Total Plate Counts for 4 Trials

Table S2. Clinical Samples Total Mean Plate Counts (TPC)

Table S3. Clinical Samples Total *S. mutans* Mean Plate Counts (SmTC)

Table S4. Ratio of *S. mutans* to Total Plate Counts

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duplicate for 2 prototype MS strains (*S. mutans* UA159 and *S. sobrinus* 6715) and for MS isolates from clinical saliva samples obtained from 16 children (approximate age 5 years) to determine total plate counts, and total *S. mutans* count. Selected isolates (n=249) from all of the five media from 5 saliva samples were further confirmed as *S. mutans* with real-time PCR then subsequently evaluated qualitatively with rep-PCR for genotype determination.

All media resulted in variable enumeration with no significant difference in MS counts. MS prototype strains grew well on all five media; clinical isolates demonstrated more variability in counts but no overall significant differences were found. MSB demonstrated comparable ability to grow *S. mutans* but allowed for more non-*S. mutans* growth. All 5 media identified a consistent predominant genotype by rep-PCR. Recovery of minor genotypes was not inhibited by media type.

Keywords

genotypes; rep-PCR; selective media; *Streptococcus mutans*; *Streptococcus sobrinus*; Total streptococci

1. INTRODUCTION

The mutans streptococci (MS) are primary etiological agents associated with the initiation of dental caries (Loesche, 1986). *Streptococcus mutans* and *Streptococcus sobrinus* are the two clinically relevant species of MS found in the oral cavity. These two species are typically identified and quantified in caries epidemiological and early intervention studies (Saravia et al., 2013).

Numerous selective media have been introduced for the isolation, quantitation, and characterization of MS. Typically these media are supplemented with bacitracin and sucrose as selective agents for clinically relevant streptococci (Gold et al., 1973, Hildebrandt and Bretz, 2006). However, unintentional inhibition of MS may occur, reducing counts and perhaps limiting the quantity of detection and isolation of some strains (Hildebrandt and Bretz, 2006, Tanzer et al., 1984). Initially, mitis salivarius with bacitracin (MSB) medium was considered a reliable selective media for isolation of MS (Gold, Jordan and Van Houte, 1973). As such, MSB medium was selected as the primary selective medium for a large-scale epidemiological study of *S. mutans* by our laboratory. However, several studies have suggested that MSB medium results in lower MS counts than other available selective media and can possibly result in false-negatives (Saravia, Nelson-Filho, Silva, De Rossi, Faria, Silva and Emilson, 2013, Schaeken, 1986, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984, Van Palenstein Helderman et al., 1983, Wan et al., 2002).

In this study, five selective media are evaluated: mitis salivarius-bacitracin (MSB), mitis salivarius—kanamycin—bacitracin (MSKB), glucose—sucrose—tellurite—bacitracin (GSTB), trypticase soy—sucrose—bacitracin (TYS20B), and tryptone—yeast—cysteine—sucrose—bacitracin (TYCSB) (Gold, Jordan and Van Houte, 1973, Kimmel and Tinanoff, 1991, Schaeken, 1986, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984, Van Palenstein Helderman, Ijsseldijk and Huis in 't Veld, 1983). Detailed descriptions of these

media can be found in the literature, but briefly, these media typically are supplemented with bacitracin and high concentrations of sucrose that act as selective agents for clinically relevant MS. Sucrose allows MS to be distinguished while inhibiting other organisms and bacitracin is known to inhibit other oral streptococci (Gold, Jordan and Van Houte, 1973, Hildebrandt and Bretz, 2006, Schaeken, 1986). Mitis salivarius media (in MSB, MSKB) is commonly used as a base medium since it is selective for streptococci and because MS colony morphology is easily distinguished on this media (Gold, Jordan and Van Houte, 1973). Sucrose is replaced by sorbitol in MSKB and the addition of kanamycin was incorporated to enhance the activity of bacitracin (Kimmel and Tinanoff, 1991).

The purpose of this study is to evaluate these five media quantitatively and qualitatively using MS prototype strains and clinical isolates. In addition, evaluation of MSB in comparison to the other four media will be assessed.

2. MATERIAL AND METHODS

2.1. MS Prototype Strains

S. mutans UA159 and *S. sobrinus* 6715 were selected as the MS prototype strains.

2.2. *S. mutans* Clinical Strains

For clinical isolates, saliva was collected from pre-school children (n=16, approximate age 5 years) as part of an ongoing longitudinal epidemiological study of a high caries risk population located in Uniontown, AL, a small rural city. Sample collection were performed at a community health center during a scheduled follow-up visit. Inclusion criteria were that children were free of systemic disease. Human use approval was obtained from the University of Alabama at Birmingham (UAB) Institutional Review Board, with parents of participants providing informed consent, while children gave assent. Whole saliva samples were collected using sterile cotton swabs, which were then stored in 4.5 ml of reduced transport fluid (Syed and Loesche, 1972). Samples were shipped on ice to our laboratory at the University of Alabama at Birmingham School of Dentistry and stored at 4°C until processed (within 24 hours of collection).

2.3. Media & Plating

All five media were prepared as originally describe in original publications (Gold, Jordan and Van Houte, 1973, Kimmel and Tinanoff, 1991, Schaeken, 1986, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984, Van Palenstein Helderma, Ijsseldijk and Huis in 't Veld, 1983).

Prototype strains were inoculated from -80°C frozen cultures into 5 ml Todd Hewitt Broth (THB) (Becton Dickinson, Sparks, MD, USA) and incubated anaerobically (10% CO₂, 10% H₂, and 80% N₂) at 37°C for 24 hours. A 500 µl aliquot of fresh culture was transferred to 4.5 ml of THB and grown to late log phase. Optical density was determined at 600 nm (OD₆₀₀) using a Bio-Rad SmartSpec Plus spectrophotometer (Bio-Rad, Hercules, CA, USA), then adjusted to an OD₆₀₀ of approximately 1.0 with THB, which corresponds to 1×10^9 cells/mL using electronic enumeration (Childers et al., 2011). Bacterial samples were

serially diluted to 1×10^{-5} and plated in duplicate on each of the five media using a spiral plater (Spiral Systems Inc., Cincinnati, Ohio, USA) dispensing a 50 μ l volume. This process was repeated in four independent tests.

Clinical saliva samples in reduced transport fluid were vortexed for 20 seconds on medium speed prior to plating. Undiluted samples were plated in duplicate on each of the five media using the spiral plater. All plates were incubated anaerobically at 37°C for 48 hours.

2.4. Enumeration

After incubation, all plates were counted using a stereomicroscope for total plate counts (TPC) and total *S. mutans* counts (SmTC) by manual counting according to the instructions for the spiral plater (Spiral Systems Inc., Cincinnati, Ohio, USA) to enumerate the colony-forming units per ml (CFUs/ml). Briefly, a quadrant sector-count method was used to enumerate bacteria using a minimum count of 20 colonies within a quadrant. Opposite quadrants in the same sector were counted and the bacterial density was found by adding the counts of the two sectors and dividing by the sample volume corresponding to the innermost sector counted.

Plates were randomly selected to perform counts without knowledge of counts on other media. The mean plate counts were calculated using duplicate plates. For the clinical isolates, SmTC were recorded based on colony morphology of the prototype strains (Figure SF1) (McGhee et al., 1982, Schaeken, 1986, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984, Van Palenstein Helderman, Ijseldijk and Huis in 't Veld, 1983).

2.5. Genotype Evaluation

For clinical samples that demonstrated sufficient *S. mutans* growth on all five selected media (n=5), 10 presumptive *S. mutans* colonies were selected for genotype identification using repetitive extragenic palindromic polymerase chain reaction (rep-PCR) as described previously (Moser et al., 2010). Briefly, colonies were inoculated in 5 ml THB and grown anaerobically at 37°C for 48 hours. DNA was extracted and checked for purity. All isolates were verified as *S. mutans* using SYBR GreenTM PCR with sequence specific Yoshida primers before performing rep-PCR (Yoshida et al., 2003). Genotypes were determined using the DiversiLabTM (DL) system and cross-referenced with the library of known genotypes from the larger epidemiological study (Cheon et al., 2013).

2.6. Statistical Analysis

Raw counts were transformed to approximate normality by taking logarithms of counts (count plus one, i.e. for counts of zero). Ratios of counts of *S. mutans* to total counts were based on raw counts, not on transformed counts. Statistical significance was ascertained using the GLM procedure in SAS version 9.2 using a significance cut-off value of $p < 0.05$.

3. RESULTS

3.1. MS Prototype Strains

S. mutans UA159 and *S. sobrinus* 6715 grew equally well on all five media with no significant differences (Tables 1 & S1). For *S. mutans* UA159, the mean log recovery was marginally better on TYS20B (9.18 ± 0.10) than on the other media; MSB (9.16 ± 0.14), GSTB (9.08 ± 0.19), TYCSB (9.09 ± 0.12), and MSKB (9.05 ± 0.16) ($p = 0.69$). In terms of frequency, the highest counts were most often obtained on MSB than other media (2/4 replicates). When MSB was compared to the other media the difference was also non-significant ($p = 0.44$).

S. sobrinus 6715 resulted in comparable counts on all five media although at a slightly decreased level as compared to *S. mutans* UA159. The highest counts were most often observed on TYS20B (8.75 ± 0.41) than other media (2/4); MSB (8.79 ± 0.30), TYCSB (8.67 ± 0.29), MSKB (8.63 ± 0.25), and GSTB (8.64 ± 0.35) but not significantly different ($p = 0.94$) when all five media were compared. In addition, no significant difference was found when comparing MSB with all other media ($p = 0.51$).

3.2. *S. mutans* Clinical Strains

Clinical saliva samples from 16 children were processed in order to compare clinical TPC and SmTC. It is characteristic of this study population that *S. sobrinus* is rarely detected either by standard plate count or quantitative PCR as part of the larger ongoing study. Thus, since *S. sobrinus* was not detected, only SmTC are reported in this study.

Four clinical samples were not included in the final analysis since growth was either not observed ($n=1$) or minimally observed on only one media ($n=3$ for GSTB <2 colonies). The Log mean plate counts observed for TPC and SmTC are reported in Tables 1 and S2, S3 respectively. TPC are reported for 12 clinical samples but SmTC are reported for only 9 clinical samples (i.e., protocol omission in counting colonies for SmTC). The highest TPC were observed on TYS20B (7/16) followed by MSB (4/16). ANOVA analysis of the log TPC for all five media yielded no statistically significant difference ($p = 0.14$). When MSB was compared to the other media no significant difference was observed ($p = 0.32$).

Concentrations of *S. mutans*, reported as SmTC, were most frequently highest on GSTB (6/9) compared with all other media types. It was observed on MSB that 22% (2/9) of the samples did not have detectable *S. mutans* when growth was observed on other media. ANOVA analysis of the log SmTC also resulted in no significant difference between the five media for recovery of clinical *S. mutans* ($p = 0.86$). The MSB comparison was also found to be insignificant ($p = 0.59$).

The percentage of *S. mutans* isolated to TPC was used as a measure of the specificity of each media (Tables 1 and S4). The ratio was highest for TYCSB ($72\% \pm 26\%$), followed by MSKB ($70\% \pm 35\%$) and GSTB ($52\% \pm 38\%$). TYS20B ($41\% \pm 37\%$) and MSB ($29\% \pm 31\%$) had the lowest percentage of *S. mutans* colonies. Overall, TYCSB and MSKB (both 4/9) most frequently reported the highest percentage of *S. mutans* specificity followed by GSTB (3/9) and MSB (1/9). The difference in the ratio of *S. mutans* to TPC was not

significant when comparing all five media ($p = 0.07$). However, when comparing MSB to the other media the difference is significant ($p = 0.03$).

The highest counts of non-*S. mutans* were most frequently observed on MSB (5/9), TYS20B (3/9), and MSKB (1/9).

3.3. Rep-PCR Genotypes

All isolates ($n=250$) except one (from ID 571, GSTB) were confirmed to be *S. mutans* using SYBR GreenTM PCR with Yoshida primers (Yoshida, Suzuki, Nakano, Kawada, Oho and Koga, 2003). Predominate genotypes were defined as “more than 50%” of the isolates being of one genotype (Cheon, Moser, Wiener, Whiddon, Momeni, Ruby, Cutter and Childers, 2013) and were found to be consistent for all samples regardless of media used (Table 2). At least one minor genotype was observed for four of the five clinical samples and one subject had 2 minor genotypes (ID 561). Minor genotypes were found most frequently with TYCSB (5/5) followed by TYS20B (4/5), MSKB and GSTB (both 3/5). MSB reported minor genotypes less frequently (2/5).

4. DISCUSSION

Direct comparison with other published data is particularly difficult since most media comparison studies do not provide raw data but rather the summary of statistical data and these statistical approaches vary considerably by study. In addition, the selection of samples for analysis also varies by study. For instance, some studies use the non-human *S. mutans* isolates *S. rattii* (serotype a) and *S. criceti* (serotype b) which potentially skew findings when analyzed collectively with the clinically relevant serotypes (Gold, Jordan and Van Houte, 1973, Hildebrandt and Bretz, 2006, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984, Van Palenstein Helderman, Ijsseldijk and Huis in 't Veld, 1983). This is particularly important in the case of MSB since growth of serotype a is not supported.

Some studies provide data only on clinical isolates without clinical controls which is a concern since other factors such as variability of ingredient quality of the culture media, methodology (plating method, incubation conditions), and technique (plate counting, plate randomization) can affect final counts (Dasanayake et al., 1995, Sanchez-Perez and Acosta-Gio, 2001, Schaeken, 1986). The use of prototype strains provides a baseline for comparison that is essential when so many variables can affect the outcome. In addition, the method for validation of isolates of *S. mutans* was typically biochemical, which is accepted practice but not infallible, particularly in the case of clinical isolates (Petti et al., 2005, Ruoff et al., 1982). It should be noted that none of the studies reviewed reported on accuracy of colony selection or if counts were adjusted to account for isolates that failed confirmation testing. All isolates in the present study were confirmed by PCR using *S. mutans* specific primers instead of biochemical testing.

4.1. MS Prototype Strains

All five media were comparable for enumeration of prototype MS strains *S. mutans* UA159 and *S. sobrinus* 6715 ($p = 0.69$ and 0.94 , respectively). When MSB is compared with all other media for the prototype strains no statistically significant difference was observed ($p =$

0.51 and 0.44, respectively). The observed decrease counts for *S. sobrinus* may be due to a difference in cell densities between *S. mutans* and *S. sobrinus*, possibly due to agglutination. Both samples were grown to an $OD_{600} \approx 1.0$, which for *S. mutans* equals approximately 1.0×10^9 cells using electronic enumeration (Childers, Osgood, Hsu, Manmontri, Momeni, Mahtani, Cutter and Ruby, 2011). However, the cell density for *S. sobrinus* has not been determined using electronic enumeration. Selectivity of the media used is not suspected to be the cause of the decreased counts, since lower counts were observed on all the media evaluated.

The finding that *S. sobrinus* grows equally well is noteworthy since some literature suggests that recovery of *S. sobrinus* (serotype d) is inhibited on MSB media (Schaecken, 1986, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984). However, a review of the literature indicates these statements are broad, confusing MSB with another media containing sulfasoxazole. The media containing sulfasoxazole inhibits growth of serotype d (Little et al., 1977). MSB media does not contain sulfasoxazole and will support the growth of serotype d. Several studies present data that supports the current finding that *S. sobrinus* grows well on MSB (Gold, Jordan and Van Houte, 1973, Kimmel and Tinanoff, 1991, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984). It should also be noted that *S. sobrinus* is not reported in the present study for clinical samples because the Uniontown study sample population rarely resulted in recovery of *S. sobrinus* (<0.003%). Although MSB is the selective media used in the Uniontown study, a very rare recovery of *S. sobrinus* in this population has been confirmed using whole saliva samples analyzed by quantitative PCR, a more sensitive detection method, in addition to standard plate counting (unpublished).

4.2. *S. mutans* Clinical Strains

Mean log counts were variable for clinical samples on all five media but variation was not statistically significant indicating all five media may be comparable for enumeration of SmTC for clinical isolates. The highest level of non-*S. mutans* were reported most frequently on MSB (5/9) and TYS20B (3/9), which may indicate these media are less specific for selection of *S. mutans*. It appears that MSB is as good at supporting *S. mutans* growth as any of the other media tested, but not quite as good at suppressing non-*S. mutans*. This may be an issue if confluent background organisms limit resources available or produce byproducts that may inhibit *S. mutans* growth. In addition, MSB demonstrated potential to report false negatives (2/9 samples). However, it is noteworthy that in these cases the SmTC for other the media were remarkable low.

In evaluating the sensitivity of each media using *S. mutans* to TPC ratios, it was observed that differences were approaching significance ($p = 0.07$) (Table 1 and S4). This value is slightly higher than the p -value reported by Wan ($p < 0.01$) (Wan, Seow, Walsh and Bird, 2002). However, when MSB was compared to the other media, the MSB effect was negative and there was a significant difference ($p = 0.03$) indicating that MSB is less effective at distinguishing *S. mutans* from other oral streptococci (i. e. less selective). However, in practice, when growth of non-*S. mutans* is observed on MSB, is not a major issue in that the

morphology of MS colonies are distinctive on MSB, and can be successfully managed by confirming selected colonies by SYBR GreenTM PCR or biochemical analysis.

4.3. Rep-PCR Genotypes

This is the first study to evaluate recovered MS strains using rep-PCR to evaluate genotype strains recovered. The use of rep-PCR adds a qualitative aspect to this study, the intent of which was to determine whether inhibition/selection of certain genotypes occurs with different media. Using rep-PCR it was found that the same predominate genotype was observed on all five media for each of the five clinical strains isolates (n=249) selected for genotyping. This suggests that isolation of a predominate genotype would be successful on any of these media. No evidence of a selection bias for any of the media tested was observed for predominate genotypes.

Isolation of minor genotypes occurred less frequently or not at all depending on media used. For instance, the second most common genotype observed was recovered on at least 4 of the five media tested in 3 of the 4 cases. It is possible that the recovery of minor genotypes occur in less frequency due to limited number of isolates selected (10 colonies per plate) or selection bias. Growth for all 7 genotypes reported in this study was observed with all five media when subsequently plated (data not shown). These findings indicate that the failure to recover some genotypes on some media in the present study was not due to differential selectivity of the media.

It should be noted, that while selection of isolates from MSB resulted in the lowest recovery of minor genotypes in this study, this was not due to inhibition of these genotypes on MSB media. In addition to the finding that all 7 genotypes grew on all 5 media, all of the reported minor genotypes in the present study have been detected from samples of the same subjects in the larger Uniontown epidemiological study using MSB. This suggests that the differential observed in the recovery of minor genotypes on MSB maybe due to the probability of selection of rare genotypes or the limited number of isolates selected (n = 10). The selection of 10 isolates is based on earlier analysis that determined that 10 isolates had a 95% probability of identifying up to 3 genotypes (Cheon et al., 2011). It is possible that either of these reasons may account for the variable recovery of minor genotypes for the other media as well. In addition, there appears to be no correlation between the SmTC of the five media and the minor genotypes observed indicating that the bias is not likely attributed to lower overall SmTC. Since the predominate genotype recovered was consistent regardless of media used, this would seem to suggest that variability of minor genotypes recovered is not due to media selectivity (Cheon, Moser, Wiener, Whiddon, Momeni, Ruby, Cutter and Childers, 2013). The results found with MSB as compared to the larger Uniontown study further support this possibility. However, sample size was small for this study and further study is required to determine if recovery of minor genotypes are media dependent or a result of sampling efficiency.

A review of the literature evaluating the selective media available for the isolation of MS suggests there are variations in colony counts depending on the media employed and the results of which media is best vary between different studies. None of the media was observed to result in statistically significant recovery of MS while simultaneously inhibiting

the growth of background organisms. It is suggested for accurate quantitation of MS in epidemiological studies and caries assessments that multiple media should be used if traditional culture methods are to be employed (Hildebrandt and Bretz, 2006). Alternately, traditional culture methodology may be supplemented or replaced with molecular testing methods such as quantitative PCR (Childers, Osgood, Hsu, Manmontri, Momeni, Mahtani, Cutter and Ruby, 2011, Hildebrandt and Bretz, 2006).

4.4. Limitations

It is possible that the SmTC may under represent the actual number of *S. mutans* since these counts were based on UA159 prototype strain morphology. However, confirmation of *S. mutans* was performed using SYBR Green™ PCR for selected isolates with 249 of 250 isolates being correctly identified representing a 99.6% accuracy rate in selection of *S. mutans* colonies based on morphology alone. Another possible limitation is the use of PCR to confirm identify *S. mutans*, which is subject to accuracy of the primer selection. A BLAST search of the primers used in this study was performed (October 2013) and were specific for only *S. mutans* at that time.

The robustness of the clinical data reported in this study may have been improved by performing replicates of plated samples in triplicate instead of duplicate. However several studies have used the approach of duplicate plating (Gold, Jordan and Van Houte, 1973, Tanzer, Borjesson, Laskowski, Kurasz and Testa, 1984, Van Palenstein Helderma, Ijsseldijk and Huis in 't Veld, 1983). Duplicate plating was selected for this study because it is the standard method by which all clinical samples are currently processed in the larger longitudinal study in Uniontown. Additionally, the clinical sample size of the current study is small but comparable to another published study (Wan, Seow, Walsh and Bird, 2002).

5. Conclusions

In this study, the five selective media currently available for the enumeration of mutans streptococci media resulted in variable enumeration with no statistically significant differences. Quantitative analysis by standard plate count using both prototype control strains and clinical strains grew comparably on all five media. MSB, when compared to other media, demonstrated comparable ability to grow but not select for MS; although it is noteworthy that colony selection based on morphology remained an effective method for isolates/genotype selection. Qualitative analysis by rep-PCR determined that the predominate genotype grew equally well on all media tested. Minor genotypes varied in recovery rates but this effect may be due to selection probability of rare genotypes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

DL	DiversiLab™
GSTB	glucose, sucrose, tellurite, bacitracin media
MS	mutans streptococci
MSB	mitis salivarius-bacitracin media
MSKB	mitis salivarius, kanamycin, bacitracin media
OD	optical density
rep-PCR	repetitive extragenic palindromic polymerase chain reaction
SmTC	<i>S. mutans</i> total plate count
THB	Todd Hewitt Broth
TPC	total plate count
TYCSB	tryptone, yeast, cysteine, sucrose, bacitracin media
TYS20B	trypticase soy, sucrose, bacitracin media

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Highlights

- Enumeration of mutans streptococci on 5 media exhibited no significant variation.
- Prototypes *S. mutans* UA159 and *S. sobrinus* 6715 grew equally well on all 5 media.
- Predominate genotype recovered was the consistent for all media types.
- Genotype recovery was not media dependent.

Summary Statistics for Log Mean Bacterial Enumeration of Mutans Streptococci on Five Media. UA159 is *S. mutans* prototype strain. 6715 is *S. sobrinus* prototype strain.

Table 1

Group	MSB	MSKB	GSTB	TYS20B	TYCSB	p value ^a	p value ^b
Control UA159	9.16 ± 0.14	9.05 ± 0.16	9.08 ± 0.19	9.18 ± 0.10	9.09 ± 0.12	0.69	0.44
Control 6715	8.79 ± 0.30	8.63 ± 0.25	8.64 ± 0.35	8.75 ± 0.41	8.67 ± 0.29	0.94	0.51
Clinical TPC	3.71 ± 0.97	3.06 ± 1.45	3.64 ± 0.93	3.80 ± 0.90	2.67 ± 1.80	0.14	0.32
Clinical SmTC	2.76 ± 1.78	3.01 ± 1.64	3.36 ± 1.14	3.22 ± 1.23	2.67 ± 1.79	0.86	0.59
Clinical Ratio	29% ± 31%	70% ± 35%	52% ± 38%	41% ± 37%	72% ± 26%	0.07	0.03 ^c

TPC is Total Plate Count. SmTC is *S. mutans* total count.

^a p-value for all 5 media,

^b p-value for MSB media compared to other media and

^c significance. Standard deviation is for 4 replicates for control strains and 2 replicates for clinical strains. Clinical samples did not detect *S. sobrinus*.

Table 2
S. mutans rep-PCR genotype counts recovered from clinical isolates on five different media.

		Rep-PCR Genotypes							
ID	Media	G-1	G1a	G-7	G-9	G-13	G-18	G-22	
553	GSTB		2				8		
	MSKB		1				9		
	MSB		4				6		
	TYS20B		2				8		
	TYCSB		1				9		
561	GSTB		8					2	
	MSKB		9					1	
	MSB		8					2	
	TYS20B		7	3					
	TYCSB		6	1				3	
562	GSTB	10							
	MSKB	10							
	MSB	10							
	TYS20B	9		1					
	TYCSB	8		2					
571	GSTB					9			
	MSKB					10			
	MSB					10			
	TYS20B					10			
	TYCSB					10			
590	GSTB			1			9		
	MSKB			1			9		
	MSB						10		
	TYS20B			3			7		
	TYCSB			2			8		

Predominate genotype is highlighted. G1a is a subtype of G1.