Comparative Recovery of *Streptococcus mutans* on Ten Isolation Media

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Received for publication 1 February 1977

The ability of *Streptococcus mutans* (Bratthall serotypes *a* through *e*) to grow on 10 isolation media was examined. The number and morphology of the colonies were observed to vary on different media. The use of blood-sucrose media consistently produced the highest recoveries. Mitis salivarius agar (MS) had higher recovery values than modified medium 10 (MM10SB), Trypticase-yeast extract-cystine medium (TYC), or MS with 1% tellurite (MST). MST with 40% sucrose (MS40S), MST with 20% sucrose and 0.2 U of bacitracin per ml (MSB), and Carlsson medium with 1% sulfasoxazole (MC), media formulated for the selection of *S. mutans*, were the most inhibitory for all serotypes. The morphology of several *S. mutans* strains was atypical on MC and MS40S, making positive identification difficult. Absence of growth of serotype *a* strains on MSB and serotype *d* strains on MC were the two major differences observed among the serotypes. Results are discussed in terms of the difficulties in making quantitative determinations from cultural data.

Studies investigating the association of *Streptococcus mutans* with dental caries have directed attention to methods for the isolation of this organism from clinical specimens. Isolation is usually accomplished by culturing samples on media containing sucrose, which results in the formation of colonies with a characteristic morphology. Results of several investigations (5, 8, 14) indicate that the percentage of recovery for *S. mutans* varies, depending in part, at least, on the medium used.

The results of human population studies (5, 9, 11, 12; J. D. de Stoppelaar, Ph.D. thesis, Drakkerij Flinkwijk, the Netherlands, 1971) demonstrate that serotype *c* is the most prevalent of the Bratthall (1) serotypes. Evidence indicates that the apparent prevalence of serotypes may be influenced by the isolation medium used. Serotype *d* strains have been reported to grow poorly on Carlsson sulfonamide media (5, 6, 11), and several stock strains of serotype *a* have failed to grow on MSB agar (5, 13).

The purpose of the present study was to examine the ability of *S. mutans* to grow on 10 isolation media and to determine whether any of the serotypes were selectively inhibited.

MATERIALS AND METHODS

The ability of *S. mutans* to grow on the following media was determined: (i) BA-1, Trypticase soy agar (Baltimore Biological Laboratories, Cockeysville, Md.), 5% defibrinated sheep blood, and 1% sucrose;

(ii) BA-5, Trypticase soy agar, 5% defibrinated sheep blood, and 5% sucrose; (iii) MS, mitis salivarius agar (Difco Laboratories, Detroit, Mich.); (iv) MST, MS, 1% Chapman tellurite (Difco) (4); (v) MS40S, MS, 1% Chapman tellurite, and 40% sucrose (total concentration) (8); (vi) MSB, MS, 1% Chapman tellurite, 20% sucrose (total concentration), and 0.2 U of bacitracin per ml (The Upjohn Co., Detroit, Mich.); (vii) TYC, Trypticase (Casitone [Difco]), yeast extract cystine (Baltimore Biological Laboratories) (J. D. de Stoppelaar, Ph.D. thesis); (viii) MM10SB, modified medium 10, with 5% sucrose and 2% sheep blood (10); (ix) MC, Carlsson medium, 0.1% sulfasoxazole (Gantrisin, Roche Laboratories, Nutley, N.J.) (2); (x) MC(.005), Carlsson medium, 0.005% sulfasoxazole (3, L. A. Thomson, doctoral dissertation, University of Michigan, Ann Arbor, 1970).

All media were prepared within the same week according to manufacturer's or developer's instructions and stored at 4°C. Media were used for a period of up to 1 month, except for MSB, which was prepared fresh weekly. Plates were stored in plastic bags to minimize evaporation.

MC media were used containing sulfonamide concentrations of 0.1%, as originally described (2), and 0.005%, which the results of previous work (16) indicated was closer to the concentration permitting growth of most *S. mutans* strains. Because the sulfonamide (sulfadimetine [Elkosin], CIBA-GEIGY Limited, Basel, Switzerland) used by Carlsson (2) is not readily available in the United States, sulfasoxazole was used in the preparation of the MC media.

The 18 strains of *S. mutans* listed in Table 1 were used in this study. Tubes containing 10 ml of Todd-Hewitt broth were inoculated with 0.1 ml of a pure culture of *S. mutans* and incubated for 20 h at 37°C.

A 3-ml portion of the broth suspension was sonically dispersed for 15 s at 20 W with a Branson W185 sonifier (Heat Systems-Ultrasonics, Inc., Plainview, N.Y.) and special microtip. Dilutions producing colony-forming units within the 30 to 300 range were calculated from standard turbidity curves read at 660 nm with a Beckman model 2S spectrophotometer. Portions, 0.1 ml, were inoculated onto five replicate plates of each of the 10 media investigated. Diluting and plating procedures were performed under aerobic conditions. Plates were then incubated in Gas-Pak (Baltimore Biological Laboratories) jars for 72 h, after which colonies were counted and mean values were calculated for each medium.

Colonies were photographed with a Bausch and Lomb Stereozoom 7 microscope equipped with a Polaroid camera and Polaroid 105 film. All plates were illuminated with reflected light only. Magnification was identical for all colonies photographed.

To facilitate comparison of media and to determine differences of statistical significance, mean counts were standardized as a percentage of the maximum mean count. The resulting standardized percentages were compared by using the Duncan multiple comparison test ($P = 0.05$). The counts were analyzed a second time after logarithms ($\log_{10}$) were taken to improve the homogeneity of the variances. Only those differences among media that were significant both in the untransformed analysis and in the log-transformed analysis were declared significant. One strain representing each serotype

was examined on two separate occasions to estimate the amount of variation inherent in the experimental methods.

The ultimate goal in comparing the growth of pure cultures on different media is to be able to interpret the results as an indication of what occurs when plaque is studied. The examination of plaque was not practical, because samples containing relatively high numbers of the individual serotypes were not readily available. An additional experiment was performed in which representative strains from each serotype were introduced into plaque samples that were free of *S. mutans* as determined by cultural and fluorescent-antibody (FA) methods. These mixtures were then dispersed and plated with the same methods as those used to study the growth of pure cultures.

RESULTS

The results of the pure culture experiment are presented in Table 2. The percent recovery value beneath each medium abbreviation is a mean value based on the individual strains within each serotype. A line underscoring any two media indicates that the differences from one to another are not statistically significant ($P = 0.05$). When the results of the individual strains are grouped according to serotype, the recovery patterns for the 10 media appeared quite similar. For all serotypes, the blood-sucrose media (BA-1, BA-5) produced the highest recoveries, with little difference between the two levels of sucrose. MST, MM10SB, and TYC had lower recoveries than MS. MS40S, MSB, and the two MC media, all of which are selective for *S. mutans*, were also the most inhibitory. There was little difference between the two levels of sulfasoxazole tested. It can be observed from Table 3 that some strains from

TABLE 1. List of strains according to serotype

Serotype				
a	b	c	d	e
AHT	Fa-1	10449	SL1	LM7
E49	BHT	GS5	K1R	V100
3720		JC1	B13	B2
OMZ61		JC2	6715	B14

TABLE 2. Media results presented as the percent recovery of the maximum mean count

Serotype	Medium									
a	<u>MSB</u>	<u>MS40S</u>	<u>MC</u>	<u>MC(.005)</u>	<u>MM10SB</u>	<u>TYC</u>	<u>MST</u>	<u>MS</u>	<u>BA-1</u>	<u>BA-5</u>
	0	6	10	13	44	55	57	77	94	94
b	<u>MSB</u>	<u>MC</u>	<u>MC(.005)</u>	<u>MS40S</u>	<u>MST</u>	<u>TYC</u>	<u>MM10SB</u>	<u>MS</u>	<u>BA-5</u>	<u>BA-1</u>
	4	14	21	22	48	54	60	73	94	98
c	<u>MC(.005)</u>	<u>MSB</u>	<u>MC</u>	<u>MS40S</u>	<u>TYC</u>	<u>MST</u>	<u>MM10SB</u>	<u>MS</u>	<u>BA-5</u>	<u>BA-1</u>
	13	15	16	20	39	44	56	60	95	96
d	<u>MC(.005)</u>	<u>MC</u>	<u>MSB</u>	<u>MS40S</u>	<u>MM10SB</u>	<u>TYC</u>	<u>MST</u>	<u>MS</u>	<u>BA-1</u>	<u>BA-5</u>
	0	0	20	24	39	42	57	63	89	97
e	<u>MC(.005)</u>	<u>MSB</u>	<u>MS40S</u>	<u>MC</u>	<u>TYC</u>	<u>MM10SB</u>	<u>MST</u>	<u>MS</u>	<u>BA-1</u>	<u>BA-5</u>
	15	21	24	26	53	58	67	77	94	99
a-e ^a	<u>MC(.005)</u>	<u>MSB</u>	<u>MC</u>	<u>MS40S</u>	<u>TYC</u>	<u>MM10SB</u>	<u>MST</u>	<u>MS</u>	<u>BA-1</u>	<u>BA-5</u>
	11	13	14	19	48	52	55	69	94	96

^a Results for all strains tested.

TABLE 3. Range of percent recovery values for all strains tested

Medium	Serotype				
	a	b	c	d	e
BA-5	88-100	84-100	84-100	86-100	85-100
BA-1	83-100	95-100	88-100	79-100	86-100
MS	63-90	63-84	31-100	43-100	51-100
MST	35-72	36-61	15-81	37-90	41-97
MM10SB	25-52	56-70	33-83	10-66	40-70
TYC	22-83	5-82	0-83	6-74	0.3-93
MSB	0	0-9	3-32	6-64	2-53
MS40S	1-43	1-32	4-50	14-65	0.1-58
MC(.005)	0-56	0-21	0-52	0	0-33
MC	0-28	2-29	0-59	0	0.3-62

each serotype failed to grow on one or more of the media; these were usually MSB, MS40S, MC, or MC(.005). In two separate experiments, one serotype *c* strain (JC1) failed to grow on TYC agar. For a particular medium, the variation in recovery levels observed within a serotype was generally as great as that observed among strains of different serotypes. The most pronounced differences among the media were the failure of serotype *a* strains to grow on MSB and the absence of serotype *d* on either of the sulfasoxazole media.

Colonies of a given strain of *S. mutans* varied in both number and morphology on different media. Figure 1 illustrates the range in colony morphology within a single strain. The colonies of most strains appeared very small on MC and MS40S, making identification difficult in mixed culture.

When a strain of *S. mutans*, in pure culture and in plaque suspension, was grown simultaneously on the different media, results were in close agreement. Comparison of recovery levels for all media produced correlation coefficients equal to 0.87, 0.93, 0.98, 0.96, and 0.99 for the strains representing serotypes *a* through *e*, respectively. These correlation values were generally comparable to those resulting from a pure culture examined on two separate occasions (Table 4).

DISCUSSION

The present study demonstrates the effect of different media on the enumeration of *S. mutans*. When a strain of *S. mutans* was grown on several media, a considerable range was observed in the percent recovery values and in colonial morphology. For a particular medium, a wide range of results was observed not only for strains of different serotypes, but also for strains within a serotype, and occasionally for repeated experiments of the same strain. Variations in the ability of a single medium or

different media to support the growth of *S. mutans* are further evidenced in results reported by other investigators.

The most notable differences in media performance have related to the growth of *S. mutans* on MSB and MC media. The paper describing MSB (7) reported no inhibition of several *S. mutans* strains, including AHT (serotype *a*). However, other investigators (5, 13) have been unable to grow serotype *a* strains on MSB. Serotype *a* strains AHT, OMZ61, E49, and 3720 did not grow on MSB in the present study. In addition, serotype *b* strain FA-1 was completely inhibited by MSB in one of the replication experiments.

Shklair and Keene (12) observed no inhibition of strains SL1, 6715, K1R, B13, or OMZ176 when they used a medium containing sulfadimetine. Furthermore, they were able to isolate serotype *d* from 15 of 194 plaque samples with similar media. Four of seven serotype *d* plaque isolates were reported by Perch et al. (11) to be sensitive to sulfathiazole. Emilson et al. (6) noted that a higher proportion of *S. mutans* from plaque grew on MS agar than on MC. They concluded that it was probable that some *d* strains are sensitive to sulfadimetine. In a later study (5), Emilson and Bratthall reported that 13 of 22 fresh isolates and 6 culture collection strains failed to grow on MC agar. In the present experiment, strains SL1, 6715, K1R, and B13 were inhibited by media containing 0.1 or 0.005% sulfasoxazole. Furthermore, at least one strain representing serotypes *a*, *b*, *c*, and *e* failed to grow on one of the sulfasoxazole media. Because sulfonamide inhibition will be reduced by incorporation into insufficiently cooled media or into media containing trace amounts of certain nutrients, it is not surprising to see considerable variation with the results from studies that use MC agar.

From Table 2, it is evident that the addition of potassium tellurite to MS agar results in a reduction in the number of colonies. This reduction ranged from 6% for the serotype *d* strains to 25% for the *b* strains, which parallels the observation of Syed and Loesche (14), who reported a three- to fourfold decrease in *S. mutans* plaque counts on MS medium containing tellurite. In contrast, Gold et al. (7) found that the addition of tellurite to MS did not affect the growth of *S. mutans*. Furthermore, differences were not detected between *S. mutans* plaque counts on MST and blood-sucrose agar (heart infusion base). In the present study, blood-sucrose agar produced higher recoveries than MS or MST. When the data for each serotype are grouped, the difference is statistically signifi-

FIG. 1. Strain JC2 (serotype *c*) incubated 48 h (GasPak) on (A) BA-5, (B) TYC, (C) MSB, (D) MS, (E) MC and (F) MS40S. Colony size ranges from 0.15 to 1.5 mm. ($\times 24$).

cant, as illustrated in the bottom row of Table 2.

It was observed by Syed and Loesche (14) that recoveries of *S. mutans* from plaque were similar on MS and MM10SB. This observation is in contrast to the results of the present study that show pure culture counts to be higher on MS than MM10SB. The differences were statistically significant for the serotype *a* and *d* strains and the grouped data representing all of the strains tested.

The most variable results were observed on TYC agar (Table 3). Strain JC1 (serotype *c*) failed to grow, and strain B14 (serotype *e*) grew very poorly, although colonial morphology varied little on this medium.

The level of variability in cultural results prevents the unqualified recommendation of a single medium for isolating *S. mutans*. Although the blood-sucrose media gave the highest recoveries in this study, other factors must be considered. Colony morphology can vary

TABLE 4. Comparison of percent recovery values for repeat runs of representative strains of *S. mutans*

Strain	% Recovery in medium:										<i>r</i> ^a
	BA-5	BA-1	MS	MST	MM10SB	TYC	MSB	MS40S	MC(.005)	MC	
AHT(1)	88	100	73	68	51	22	0	13	40	22	0.89
AHT(2)	100	83	90	72	44	58	0	12	23	21	
Fa-1(1)	98	100	73	36	70	29	0	1	0	2	0.87
Fa-1(2)	84	100	63	48	56	79	4	31	11	21	
JC2(1)	84	100	58	52	48	61	32	29	23	37	0.87
JC2(2)	99	100	80	51	78	72	11	44	37	44	
SL1(1)	100	83	64	60	10	43	6	14	0	0	0.85
SL1(2)	100	87	92	90	66	74	51	49	0	0	
LM7(1)	99	100	51	41	50	39	10	12	0	1	0.72
LM7(2)	95	92	100	95	65	93	44	12	31	62	

^a *r*, Coefficient of correlation.

considerably from medium to medium, and on blood-sucrose agar some strains of *S. mutans* produce atypical colonies, especially when the plate is crowded. There is also the possibility that the presence of *S. mutans* will be masked by the overgrowth of other organisms. Samples containing few *S. mutans* are likely to be found negative even on media giving the best recoveries. The colony-forming units of *S. mutans* would be lost in the dilution necessary to produce an uncrowded plate. In this type of situation, it may be advisable to use selective media such as MSB, MC, or MS40S, provided their deficiencies are fully realized. If sample dilution is minimal, the inhibitory effect of these media may be reduced. This rationale is supported by the study of Emilson et al. (6), who detected *S. mutans* in all 36 plaque samples examined by FA methods. On MS agar, 8 of the 36 samples were negative for *S. mutans*, whereas on MC agar, 7 of the negative samples were positive. The present study indicates that the sole use of either MSB or MC is limited because of their failure to support the growth of selected serotype *a* or *d* strains, respectively. Furthermore, the atypical morphology observed with several strains on MS40S and MC could result in the failure to recognize a positive sample.

If the levels of recovery of *S. mutans* from plaque parallel the results of pure culture experiments, it would be expected that the percentage of *S. mutans* in the total cultivable flora would vary with the choice of a medium. The study of Emilson and Bratthall (5) supports this premise, although the differences they observed in *S. mutans* plaque counts among several media were not as great as differences observed with pure cultures on the same media.

In the present study, the number of *S. mutans* colonies on a particular medium was essentially the same whether pure cultures or plaque suspensions were used as the inoculum.

The results of this study demonstrate the variation in recoveries of *S. mutans* on different media and the limitation this approach places on making quantitative determinations from cultural results. A combination of FA and cultural methods is currently used in this laboratory for the detection and isolation of *S. mutans* from clinical specimens. The media employed are the two that consistently gave the highest recoveries in this study, i.e., BA-5 and MS. In situations where a specimen is negative culturally but positive by FA, isolation is attempted by direct plating onto one or more of the selective media, depending on the serotype(s) identified by the FA technique.

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