

Growth of *Streptococcus mutans* on Various Selective Media

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The ability of *Streptococcus mutans* to grow on mitis-salivarius (MS) agar, MC agar, mitis-sucrose-bacitracin (MSB), BCY agar, and MM10 sucrose agar was studied. Batch cultures of *S. mutans* serotype *a* demonstrated no growth on MSB agar. Certain serotype *d* and *g* strains did not grow on MC agar. The yield for most strains of other serotypes on these selective media was lower compared with that on MS agar. The number of total colony-forming units on BCY and MM10 sucrose agar was similar to the blood agar results. Similar data were obtained when fermenter-grown strains, harvested in the middle or the end of the logarithmic growth phase, were used for inoculation of the various media. Enumeration of *S. mutans* from plaque samples plated on MC and MSB agar yielded about 75% of the counts obtained on MS or the nonselective medium. When the proportions of *S. mutans* were expressed as a percentage of the total cultivable flora, the selective media (MC and MSB agar) showed approximately 10% lower values than the MS, BCY, and MM10 sucrose agar.

During the last 15 years, *Streptococcus mutans* has been a subject of particular interest in dental caries research. Consequently, many attempts have been made to develop rapid and sensitive methods for its detection. The use of mitis-salivarius (MS) agar has dominated various cultural techniques. On this medium, *S. mutans* has a characteristic colonial morphology, which permits its differentiation from other oral streptococci (4, 7, 12; A. L. Thomson, D.P.H. thesis, Univ. of Michigan, Ann Arbor, 1970). However, in many instances, identification by the morphological criterion requires considerable experience. When used in extensive clinical investigations, this procedure can be time consuming and tedious. Likewise, it does not permit the detection of *S. mutans* present in low numbers relative to other streptococci. These liabilities, as well as others, initiated the development of selective media, namely, MC agar (3) and mitis-sucrose-bacitracin (MSB) agar (9).

Clinical studies that quantitatively relate *S. mutans* to the total number of recoverable bacteria must use a nonselective medium (i.e., blood agar) for enumeration of the total flora if MS, MC, or MSB agar has been used to assess the *S. mutans* population within the sample. This is not necessary when BCY (10) or MM10 sucrose agar medium (17) is used. These media (BCY and MM10) are nonselective, but *S. mutans* can be identified on the basis of its colonial morphology.

The purpose of this study was to compare the

growth ability of *S. mutans* on the various media mentioned (Table 1). Since different serotypes of *S. mutans* do not have identical biochemical characteristics, data are also presented regarding the ability of these media to support growth of strains of various serotypes.

Microorganisms. Twenty-six strains of the culture collection at Odontologiska kliniken in Göteborg and representing serotypes *a* through *e* (1) were selected. Strains OMZ 175 (serotype *f*) and K1-R (*g*) (16) were also used. In addition to these strains, 22 fresh oral isolates of the *d* and *g* serotypes were included.

Pure culture studies. One strain of each serotype (3720, *a*; BHT, *b*; KPSK2, *c*; B13, *d*; LM 7, *e*; OMZ 175, *f*; K1-R, *g*) were grown in a streptococcus broth (11). The cultivations were performed under continuous stirring at 37°C in a fermenter (FG-500, Biotec AB, Sweden) containing 500 ml of broth. The pH was stabilized at 6.8 with 5 N NaOH by means of an automatic titrator (TTT 11 and Autoburette ABU 13, Radiometer, Copenhagen, Denmark). At the start of each cultivation, 50 ml of a 15-h broth culture of the organism was introduced into the fermenter. Samples were obtained twice: (i) in the middle of the logarithmic phase and (ii) immediately after termination of the acid production, as indicated by titrigraph (SBR 2, Radiometer, Copenhagen)-monitored consumption of NaOH. The cells were centrifuged at 3,000 × *g*, washed once in 0.05 M phosphate buffer (pH 7.3) with 0.4% KCl, and diluted in 10-fold steps in the same buffer solution to 10⁻⁶. Samples of 0.1 ml of the final dilution were plated in duplicate on the various agar media listed in Table 1. The plates were incubated in an atmosphere of 95% N₂ and 5% CO₂ for 48 h at 37°C. For two

TABLE 1. Agar media used for enumeration and identification of *S. mutans*

Agar medium	Remarks
MS (Difco)	<i>S. mutans</i> identified on basis of colonial morphology (4, 7, 12)
MC (3)	Selective medium containing sulphadimetine (Elkosine, CIBA) 0.1%
MSB (9)	Selective medium containing 0.2 U of bacitracin per ml. (A·L, Norway) and 20% sucrose
BCY (10)	Nonselective brain heart infusion medium containing Casitone-cysteine-hydrochloride and 5% horse blood; <i>S. mutans</i> identified on basis of ridged colonial morphology (10)
MM10-sucrose (17)	Nonselective medium containing Trypticase, yeast extract, 5% sucrose, and 2% horse blood; <i>S. mutans</i> identified on basis of colonial morphology (14)
Blood	Nonselective medium consisting of blood agar base no. 2 (Oxoid) with 5% defibrinated horse blood

of the strains, BHT (*b**nd KPSK2 (*c*), the entire procedure was performed three times and new batches of media were prepared each time.

The 26 *S. mutans* strains, serotypes *a* through *e*, were grown in the broth without stabilized pH. The cells were harvested after 16 h, washed, diluted, and plated on the agar media as described previously. The 22 fresh *d* and *g* isolates were also cultivated under these conditions but were only plated on MS and MC agar. As sulphadimetine is the selective agent in the MC agar, the fresh *d* and *g* strains were tested for sensitivity to sulfonamide (5) using sensitivity tablets (A/S Rosco, Denmark) on blood agar plates. A zone of inhibition greater than 25 mm in diameter after overnight incubation was considered to be a positive reaction.

Plaque samples. Plaque material from 10 subjects known to harbor *S. mutans* was collected by means of a dental carver and transferred to 2 ml of reduced transport medium (17). After agitation in a Whirlmixer (Scientific Industries Ltd., England) for 1 min, 1-ml samples were diluted in the buffer described previously. From each of the 10^{-4} and 10^{-5} dilutions, two portions of 0.1 ml were plated on the different agar media. The total number of colonies and colonies resembling *S. mutans* were enumerated after 48 h of anaerobic incubation. The pair of plates giving a reasonable number of colonies (30 to 300 per plate) was selected. Representative *S. mutans* colonies, as well as those with questionable morphology, were isolated and checked for identity utilizing immunofluorescence (2).

In the fermenter experiments, no major differences were observed between cells harvested in the middle or the end of the logarithmic

growth phase (Table 2). The number of colonies on the BCY and MM10 sucrose media were similar to the blood agar values. Slightly lower results were observed on the MS and MC agar, whereas the MSB plates yielded the lowest number of colonies. Fermenter-grown strains of the other serotypes demonstrated similar results. The serotype *d* (B13) and *g* (K1-R) strains did not grow on MC agar. Our isolate of AHT (*a*) did not grow on MSB agar.

When *S. mutans* strains were cultivated under batch conditions, none of the five serotype *a* isolates grew on MSB agar and none of the six serotype *d* strains grew on MC agar. To further assess the growth ability of the MC agar, fresh *d* and *g* isolates were tested. Nine of these strains grew on MC agar with an average yield of 73% of that obtained on MS agar (100%), whereas, 13 strains did not grow on MC agar. Sensitivity testing demonstrated that 11 of the strains, including the nine strains that grew on the MC agar, were resistant to sulfonamide.

The results of the plaque sample analysis are presented in Table 3. The mean total number of colonies on the two nonselective media was 92% for BCT and 80% for MM10 sucrose as compared to the blood agar yield (100%). In comparing the number of *S. mutans*, the BCY and MM10 sucrose approximated the MS agar yield (100%), whereas the MC and MSB values were about 75% of the MS yield.

The percentages of *S. mutans* in the plaque samples were calculated in two ways. The MS, MC, and MSB values of *S. mutans* were compared to the growth on blood agar plates. The

TABLE 2. Mean and range of colonies of *S. mutans* strains BHT and KPSK2 after triplicate culturing in a fermenter

Strain	Agar-medium	I ^a		II ^a	
		%	Range	%	Range
BHT	Blood ^b	100		100	
	MS	84	66-96	89	77-111
	MC	61	13-88	82	71-95
	MSB	12	3-17	32	26-40
	BCY	91	83-99	95	85-104
	MM10	99	96-102	92	78-100
KPSK 2	Blood ^b	100		100	
	MS	70	66-75	77	71-84
	MC	67	49-87	90	82-98
	MSB	16	10-23	14	11-18
	BCY	110	101-120	93	91-95
	MM10	98	96-99	98	84-122

^a Samples for culturing were taken in the middle (I) and at the end (II) of the logarithmic growth phase.

^b The number of colonies on blood agar was regarded as 100%.

TABLE 3. Mean and range of total number of colony-forming units (CFU) and *S. mutans* on the various agar-media and percentage of *S. mutans* in plaque samples from 10 subjects

Agar medium	Total CFU	Range	Total no. of <i>S. mutans</i>	Range	% <i>S. mutans</i>	Range
Blood ^a	100					
BCY	92	56-156	94	72-121	32.2	3.1-62.4
MM10	80	52-110	98	50-146	32.3	14.9-58.1
MS ^a			100			
MC			76	36-102		
MSB			72	43-118		
MS/blood					30.4	9.2-57.1
MC/blood					22.1	4.7-40.5
MSB/blood					20.6	4.0-38.1

^a Blood and MS values were regarded as 100%.

number of *S. mutans* on BCY and MM10 sucrose was compared to the total growth on the same plates. The results showed mean numbers varying from 20 to 32% of the total cultivable flora, with the MC and MSB yield approximately 10% lower than the MS, BCY, and MM10 sucrose values.

The pure-culture studies revealed some points of interest regarding certain characteristics of the selective media. It appeared that none of our serotype *a* isolates could be cultivated on MSB agar. This observation is in accordance with the finding (6) that strains of genetic group IV of *S. mutans* (*S. mutans* subsp. *cricketus*) fail to grow on MSB medium. None of the *d* or *g* serotypes from the culture collection demonstrated growth on MC agar. However, of the 22 fresh *d* and *g* isolates, 9 grew on the MC agar. As would be expected, none of the sulfonamide-sensitive strains were included in this group. Due to the fact that serotype *a* strains are very rare in Sweden, no fresh isolates of this serotype could be tested on MSB agar.

Culture collection strains, harvested in different growth phases, demonstrated lower yields on MSB, MC, and MS agar than on the nonselective media (Table 2). From these data one would expect similar results when studying plaque samples, but, as seen in Table 3, this assumption was only partially correct. For example, the MS agar yield approximated the BCY and MM10 sucrose values. However, the total number of *S. mutans* cultivated on MC and MSB agar was about 75% of the yield obtained on MS, BCY, and MM10 sucrose media. The recovery on MC and MSB agar, when expressed as percentage of the total viable flora, was approximately 10% lower as compared with the relative proportions obtained on the nonselective media.

Loesche and Syed (13) have pointed out that the use of selective media "is done at a price." Our results support this view. The selective

media may quantitatively underestimate plaque samples with relatively high proportions of *S. mutans*, as were used in this study (Table 3). These lower values may in part be explained by the fact that most serotypes are depressed to a certain degree by these media and in part by the total exclusion of serotype *a* on MSB agar and certain *d* and *g* strains on MC agar. However, the selective media are superior when *S. mutans* is present in very low proportions (3, 9), as in saliva samples, where *S. mutans* often constitutes less than 1% of the cultivable flora (8).

Finally, a few other characteristics of the media should be pointed out. Only the BCY and MM10 sucrose agar permit the enumeration of *S. mutans* and the total flora on the same plate. Likewise, MM10 sucrose agar allows differential counting of *S. sanguis* (17), whereas MC agar also supports the growth of *S. milleri* (15).

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