Inhibition of *Streptococcus mutans* Strains by Different Mitis-Salivarius Agar Preparations

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Several *Streptococcus mutans* strains were markedly inhibited by mitis-salivarius agar manufactured by Baltimore Biological Laboratories, but little, if any, inhibition was noted using Difco Laboratories' mitis-salivarius agar. Supplementation of the basic medium with sucrose and bacitracin for specific selection of *S. mutans* resulted in suppression of representative *S. mutans* type *a* strains regardless of manufacturer.

Many studies have suggested a positive association between the microbe *Streptococcus mutans* and human tooth decay. Longitudinal studies currently in progress are attempting to relate the future development of enamel cavitation with the percentage of *S. mutans* recovered in the dental plaque microflora removed from intact tooth surfaces. This research may lead to the introduction of an *S. mutans*-based clinical laboratory caries susceptibility test that is sufficiently sensitive to identify individuals with a high probability of developing carious lesions.

There are several microbiological media available for the detection and enumeration of oral streptococci, including *S. mutans* (2, 4-6, 9); however, mitis-salivarius (MS) agar (4) appears to be the most widely used formulation because of its selective and differential characteristics. Recently, Gold et al. (5) reported that the addition of 15% sucrose and 0.2 U of bacitracin per ml of medium to the basic MS agar formulation resulted in the inhibition of all oral streptococci except *S. mutans* and some enterococci. Using MS agar and the sucrose-bacitracin-supplemented MS (MS-SB) agar in clinical experiments, we have noted occasional discrepancies between data from the two media for the recovery and enumeration of *S. mutans* from human dental plaque.

In an attempt to control or understand these variations in recovery, we plated dilute pure broth culture suspensions of *S. mutans* strains on MS agar, MS-SB agar, and Trypticase soy agar supplemented with 0.5% glucose. The data from the Trypticase soy-glucose agar were used to define 100% recovery for each strain tested. Media manufactured by Difco Laboratories (Detroit, Mich.), control 600877, and Baltimore Biological Laboratories (BBL; Cockeysville, Md.), lot E6DBKK, were evaluated. All media were prepared according to the manufacturer's

directions and were utilized within 3 days of preparation. The plates were incubated at 37 C for 48 h in an atmosphere of 80% N₂, 10% CO₂, and 10% H₂. The data in Table 1 indicate that the percentage of recovery of each pure laboratory *S. mutans* strain was greater using MS agar manufactured by Difco compared to the BBL product. Comparing recoveries on the MS-SB agar, it was found that the Bratthall type *a* *S. mutans* strains (1) were effectively inhibited by the selective medium based on either manufacturer's MS agar. In addition, the BBL-based MS-SB agar substantially reduced the recoveries of all *S. mutans* laboratory strains evaluated. Different lots of the two manufacturers' media have been tested and similar patterns of inhibition were seen, although the recovery percentages varied (data not shown).

Recovery of *S. mutans* on different MS and MS-SB agars from diluted dental plaque samples obtained from three children with active caries is presented in Table 2. The subjective criterion of colonial morphology was used for identification of *S. mutans* (3, 7, 8). *S. mutans* was recovered on both Difco MS and MS-SB agars from the three children. The total *S. mutans* colonies recognized for each patient on the Difco MS agar compares closely with the Difco-based MS-SB agar data. Using the BBL MS agar, *S. mutans* was detected in one (subject "B") of the three children. The BBL MS agar recoveries were less than 10% of those seen on Difco MS agar for the same plaque sample. Recovery of *S. mutans* from subject "B" on BBL MS-SB agar represented less than 1% of the *S. mutans* colonies detected on the Difco-based media.

There are no ready explanations for the variations in recovery of *S. mutans* on MS agar produced by the two manufacturers, since their published formulations are essentially identi-

TABLE 1. Percentage of recovery of laboratory strains of *S. mutans* on supplemented and nonsupplemented MS agar manufactured by two commercial firms

<i>S. mutans</i>		Medium				
Strain	Serotype ^a	TSA-G ^b	BBL		Difco	
			MS	MS-SB	MS	MS-SB
OMZ 61	<i>a</i>	100 (120)	65 ^c	0	91	0
AHT	<i>a</i>	100 (30)	27	0	72	0
BHT	<i>b</i>	100 (397)	36	3.0	106	83
INGBRITT	<i>c</i>	100 (189)	1.6	0	102	20
SL-1	<i>d</i>	100 (336)	5.7	3.0	87	75
OMZ 176	<i>e</i>	100 (52)	0	0	100	87

^a Bratthall grouping (1).^b TSA-G, Trypticase soy-glucose agar. Numbers in parentheses indicate average number of colony-forming units obtained from triplicate plate counts.^c Recoveries are expressed as percentages relative to the recovery of the same strain on the nonselective TSA-G medium.TABLE 2. Recovery of *S. mutans* and total oral streptococci from human dental plaque using supplemented and nonsupplemented MS agar manufactured by two commercial firms

Subject	CFU ^a on BBL medium				CFU on Difco medium			
	MS agar		MS-SB agar		MS agar		MS-SB agar	
	<i>S. mutans</i>	Total	<i>S. mutans</i>	Total	<i>S. mutans</i>	Total	<i>S. mutans</i>	Total
A	0	111	0 ^b	0	1	117	1.5	1.7
B	3.5	117	0.3	18.5	44	108	49	49
C	0	14.3	0	5.1	2.9	13.0	3.5	3.8

^a Colony-forming units (CFU) were averaged from duplicate plate counts and represent a 10⁶ dilution of the original sample.^b CFU obtained at a 10⁵ sample dilution are underlined.

cal and both contained equivalent amounts of the potential inhibitor potassium tellurite (9, 10). The data from the clinical samples (Table 2) indicate that the total colony-forming units detected on the MS agar were nearly equal for the two products. Therefore, it can be suggested that on the BBL MS agar the *S. mutans* did not form the characteristic "convex, frosted-glass" colonial morphologies (3, 7, 8) and, thus, were not detected. However, heavy inoculation of laboratory strains of *S. mutans* on BBL MS agar results in characteristic growth, and identifiable, characteristic colonies were isolated from one of the clinical samples on the BBL MS agar. During examination of the clinical samples grown on BBL MS and MS-SB agar, a number of non-*S. mutans*, small, circular, flat, brown colonies containing gram-positive cocci were encountered. These colonies were detected on Difco MS and MS-SB agar only when high concentrations of dental plaque were plated. Thus, it appears that the BBL product suppresses the growth of *S. mutans*, whereas the Difco preparation is inhibitory to an unidentified gram-positive coccus.

Gold et al. (5) reported that 0.25 U of bacitra-

cin per ml of Difco MS agar was noninhibitory to *S. mutans* AHT; however, our data indicate that 0.2 U of bacitracin per ml of either manufacturer's MS agar in combination with an additional 15% sucrose was sufficient to inhibit many of the Bratthall type *a* *S. mutans*, including AHT. Type *a* inhibition by the MS-SB medium has been noted in other laboratories (W. Loesche, personal communication; W. Lilje-mark, personal communication).

Presentation of this data is not intended to discourage the use of MS agar or to diminish the value of the MS-SB agar as a potential clinical diagnostic tool but, rather, to alert investigators to potential differences in recoveries of *S. mutans* using commercial preparations of this medium and to suggest the use of alternate methodologies as controls to verify the enumeration of *S. mutans*.

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