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Differential Recovery of *Streptococcus mutans* from Various Mitis-Salivarius Agar Preparations

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Recoveries of *Streptococcus mutans* from human dental plaque were lower when plated on mitis-salivarius agar obtained from Baltimore Biological Laboratories as compared with mitis-salivarius agar obtained from Difco Laboratories. However, no difference in recoveries of established laboratory strains of *S. mutans* was observed between these two agar preparations.

Streptococcus mutans has been implicated by many investigators to be causally associated with human dental caries (3, 6, 7, 9). Of critical importance in many of these studies was the ability to distinguish *S. mutans* from other oral streptococci by the formation of a unique type of colony on the selective differential medium mitis-salivarius (MS) agar (2, 5, 8). During a long-term study designed to evaluate the relationship between dental plaque and dental caries, differences in the recoveries of *S. mutans* from the same dental plaque sample were observed when the sample was plated onto MS agar obtained from Baltimore Biological Laboratories (BBL), Cockeysville, Md., and from Difco Laboratories, Detroit, Mich. Significantly fewer *S. mutans* were recovered using the BBL preparation. In addition, a similar discrepancy was observed between these agars when the bacitracin, high-sucrose *S. mutans* selective media described by Gold et al. (4) was utilized. Studies were therefore initiated to document and evaluate these observations.

Supracoronal dental plaque was collected from the facial surfaces of the first four permanent molars of children, aged 6 to 8 years, with sterile curettes and from the interproximal surfaces of these permanent molars and the second deciduous molars with sterile dental floss. The plaque and floss were pooled into 3 ml of iced, reduced transport fluid (11) and analyzed within 3 to 4 h as described below.

Strains of *S. mutans* serotyped according to the method of Bratthall (1) were obtained from either A. Coykendall, Great Lakes Naval Station, Chicago, Ill., or from the Forsyth Dental Center, Boston, Mass. The strains included were: serotype a, AHT, OMZ-61, HS-6; serotype b, BHT, FA-1; serotype c, GS-5, Inbritt; serotype d, 6715, SL-1; and serotype e, LM-7. In addition, fresh isolates of *S. mutans* were obtained from the plaque samples after plating as described below. These strains included S-EM, M-192, M-497, M-504, M-469, and M-489. None of these fresh isolates were serotyped.

The plaque samples were subjected to sonic oscillation for 20 s with a Branson sonifier model-S75 equipped with a microprobe, immediately serially diluted into Ringers solution, and plated in duplicate onto two different batch lots of BBL and Difco MS agar (prepared according to company specifications). Exponential phase cultures of the defined strains of *S. mutans* growing anaerobically (see below) in Trypticase soy broth (BBL) containing 0.25% yeast extract (Difco) and 2% glucose were subjected to sonic oscillation, serially diluted, and plated in duplicate onto the same media as the plaque samples. In addition, the bacteria were plated onto Gold's MS bacitracin agar made with either BBL or Difco components. Plates were stored for no more than 3 days prior to use. After inoculation the plates were incubated anaerobically in an atmosphere of 80% N_2, 10% H_2, and 10% CO_2 for 48 h at 37°C and then incubated aerobically for 24 h at room temperature before counting. *S. mutans* and other streptococcal colonies were identified using the criteria described in detail by others (2, 5, 8).

Of the 20 dental plaque samples analyzed, 6 were shown to contain detectable numbers of *S. mutans*. Five of the six samples showed 1.7-, 2.0-, 2.8-, 7.5-, and 200-fold higher recoveries of *S. mutans* on Difco MS than on BBL MS agar. One sample showed a 2.1-fold higher recovery of *S. mutans* on BBL MS agar. These observations are similar to those of R. H. Staat (10). No consistent selective difference in the recovery of *Streptococcus salivarius*, *Streptococcus sanguis*, or *Streptococcus mitis* was discernible, and the total recoveries of all streptococci on these agars were less in the BBL agar when *S.*

mutans was present in the plaque sample. No interbatch differences in the two lots of either BBL or Difco were noted.

In addition, freshly isolated strains of *S. mutans* were studied. When exponential phase cultures of these freshly isolated *S. mutans* were plated, significantly lower yields were observed on BBL MS and its respective Gold's bacitracin preparation than were found on Difco MS and its respective Gold's bacitracin preparation (Table 1).

However, when exponential phase cultures of 10 laboratory adapted strains of *S. mutans* were diluted and plated onto BBL and Difco MS agars and the Gold's bacitracin preparations, no consistent significant difference was observed in the selectivity between BBL and Difco MS agars or the different batches (Table 1). These data contrast with those of Staat (10), who noted that better recovery of laboratory strains of *S. mutans* were obtained with Difco MS. Nonetheless, in the present study, Gold's bacitracin preparations of both Difco and BBL agars completely inhibited growth of strains of *S. mutans* serotype a (HS-6, AHT, OMZ-61). In addition, growth of strains of *S. mutans* serotype b (FA-1) and serotype e (LM-7) were significantly inhibited by BBL and Difco Gold's bacitracin agars (Table 1).

TABLE 1. *Plating efficiency of Streptococcus mutans isolates on various media*

S. mutans strain	Plating media[a]			
	BBL	BBL + bacitracin	Difco	Difco + bacitracin
Fresh isolates				
S-EM	0.6	<0.01	46	49
M-192	1.4	0.8	15	11
M-497	2.2	1.5	6.4	6.1
M-504	1.8	0.3	5.1	3.3
M-469	2.1	0.5	7.8	5.1
M-489	1.4	0.8	1.5	1.3
Bratthall serotypes of stock cultures				
OMZ-61 (a)	7.1	<0.01	9.8	<0.01
AHT (a)	21	<0.01	19	<0.01
HS-6 (a)	12	<0.01	12	<0.01
FA-1 (b)	43	2.0	34	0.3
BHT (b)	34	8.0	33	19
Ingbritt (c)	43	46	57	38
GS-5 (c)	44	45	50	36
6715 (c)	62	38	68	33
SL-1 (d)	26	24	35	31
LM-7 (e)	14	4.9	14	4.6

[a] Colony-forming units per milliliter times 10^6.

BBL and Difco MS agars differ markedly in their degree of selectivity against freshly isolated *S. mutans*, with Difco giving significantly better yields than BBL. However, for laboratory-adapted strains of *S. mutans*, little or no selective differences were observed. Growth of *S. mutans* of serotype a was drastically inhibited by Gold's selective media prepared in either BBL or Difco MS agars. Thus, indiscriminate interchangeable use of these selective agars is not advised, particularly when maximum recoveries of *S. mutans* strains and differential identification between oral streptococci is required.

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