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Comparison of Selective Media for Isolation of Presumptive Group D Streptococci from Human Feces

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Pfizer Selective Enterococcus (PSE) agar, a medium containing bile, sodium azide, and esculin, was evaluated for its sensitivity and selectivity for detection and enumeration of presumptive group D streptococci in human feces. SF broth and SF broth plus agar (1.5%), representing selective media in common use, were studied simultaneously. Presumptive group D streptococci were recovered on PSE agar from the feces of all 25 subjects. No growth was observed in 8% of specimens in SF broth. No gram-negative organisms were recovered in any medium. PSE agar has the advantages of selecting out *Streptococcus bovis*, earlier appearance of distinctive reactions, and lack of requirement for special incubation temperature.

Studies of bowel flora are important in a wide variety of disorders and for establishing the natural distribution of groups or species of bacteria. In these, the use of selective and differential media is necessary to detect bacteria present in relatively small numbers. SF broth (BBL) is commonly used in many laboratories for detection and enumeration of enterococci from feces, water sources, and foodstuffs. To be selective, the broth must be incubated at 45 C. Our definition of fecal streptococci, enterococci, and group D streptococci is adopted from Hartman et al. (3) as shown in Fig. 1.

Facklam and Moody (2) recommended the use of a bile-esculin test in a medium containing 5% horse serum as the test of choice for presumptive identification of group D streptococci. This reaction correctly identified 100% of group D strains and ruled out 97% of the Viridans strains. Positive reactions were also observed with group Q strains. Isenberg et al. (4) tested a new selective medium, Pfizer selective enterococcus (PSE) agar, containing bile, sodium azide, and esculin, for the rapid detection of group D streptococci in clinical and fecal specimens. They concluded that this medium had a sensitivity equal to that of blood- or Mitis-Salivarius agar, possessed superior selectivity, and allowed presumptive identification based on esculin hydrolysis. No esculin-positive streptococci other than group D were reported. Species of group D streptococci isolated from fecal material were not indicated separately from those isolated from clinical material. The above information prompted us to confirm and extend their studies with regard to primary isolation and enumeration of various group D streptococci from human feces. The purpose of this communication is to report the results of this study.

MATERIALS AND METHODS

Five ATCC strains of streptococci (*Streptococcus durans*, 19432; *S. faecalis* var. *liquefaciens*, 13398; *S. faecium*, 19579; *S. faecium* var. *mobilis*, 14436; and *S. bovis*, 15352) were inoculated into Brain Heart Infusion broth and incubated for 18 hr at 37 C. Serial 10-fold dilutions were made of these broth cultures, and 0.5-ml samples were surface-streaked onto PSE agar and 5% sheep blood-agar plates.

Freshly voided stools from 25 normal subjects were serially diluted by 10-fold steps in 0.05% yeast extract solution. Samples (0.5 ml) of selected dilutions were inoculated on or into the following media: PSE agar, SF broth, and SF broth plus 1.5% agar. The last two media were incubated at 45 C; the former were incubated at 37 C. Cultures were observed at 18, 36, and 48 hr. Colony counts were made from the solid media and were expressed in terms of dry weight of the stool specimens. The colonial characteristics were noted and then colonies were subcultured. Isolation was carried out whenever necessary. Gram stain smears of all different colony types were prepared. Gram-positive cocci were tested for catalase; if negative, growth in 40% bile, growth at 45 C, and ability to grow and hydrolyze esculin on PSE agar (if isolated from other media) were observed. Organisms were characterized as probably

belonging to serological group D if they were capable of growth under the above conditions and hydrolyzed esculin. Streptococci that did not show the above characteristics were regarded as non-group D. Catalase-positive, gram-positive cocci were regarded as *Micrococcus* or *Staphylococcus*.

Further identification of presumptive group D streptococci was done on strains recovered from 15 of the 25 subjects. This was carried out with the following tests: growth in 6.5% NaCl, liquefaction of gelatin, hemolysis of 5% horse blood-agar pour plates, and fermentation of sorbitol, mannitol, sucrose, and lactose. Characteristics used for presumptive identification of strains were selected from those of Shattuck (6) and are indicated in Table 1.

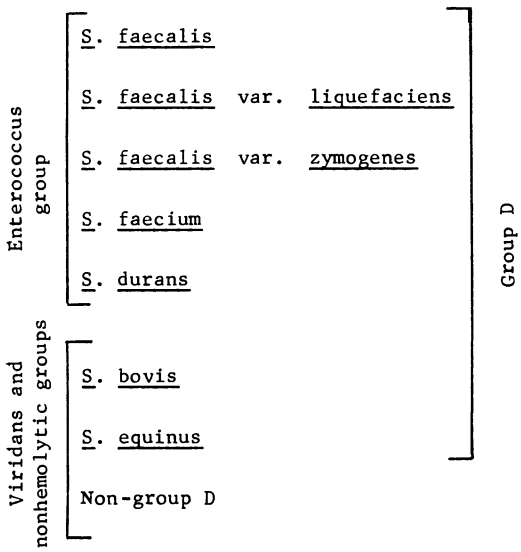

FIG. 1. *Definition of fecal streptococci.*

RESULTS

The five ATCC strains tested grew on the two media in comparable numbers within the same dilution. Reactions on PSE agar were evident within 18 hr of incubation. From normal human feces (Table 2 and Fig. 2), various species of enterococci were recovered from comparable numbers of subjects and in similar colony counts on the solid media. One or more species of presumptive group D streptococci were isolated from all specimens on the solid media. In no case did we recover more than one species from SF broth, and in two instances no growth was observed. Bacteria resembling *S. faecalis* var. *zymogenes* and *S. equinus* were not recovered on any of the media. Organisms resembling *S. bovis* were recovered only from PSE agar (on four occasions). In one case, *S. bovis* was present in higher numbers than *S. faecalis* and precluded the recovery of the latter on PSE agar. Colonies typical of group D streptococci on PSE agar were seen in all cases. Characteristically, the colony is round, entire, raised, about 2 mm in diameter, brown, and translucent with a surrounding black halo (the latter indicating esculin hydrolysis). Non-group D streptococci and micrococci or staphylococci recovered on PSE agar were easily distinguished from presumptive group D streptococci because of their inability to hydrolyze esculin. No gram-negative organisms were recovered from any of the media. Blackening of the medium by presumptive group D streptococci was always observed within 18 hr of incubation, whereas the color change of the indicator in SF broth, with or without agar, usually appeared at 48 hr or later.

TABLE 1. *Criteria used for presumptive identification of species*

Characteristic	Species						
	S. faecalis	*S. faecalis* var. *liquefaciens*	*S. faecalis* var. *zymogenes*	*S. faecium*	*S. durans*	*S. bovis*	*S. equinus*
Growth and blackening PSE agar	+[a]	+	+	+	+	+	+
Growth on 40% bile agar	+	+	+	+	+	+	+
Growth at 45 C	+	+	+	+	+	+	+
Growth in 6.5% NaCl	+	+	+	+	+	−	−
Fermentation of							
Sorbitol	+	+	+	−	−	−/+	−
Mannitol	+	+	+	+	−	−/+	−
Lactose	+	+	+	+	+	+	−
Sucrose	+	+	+	+/−	−	+	+
Liquefaction of gelatin	−	+	−	−	−	−	−
β-Hemolysis (horse blood)	−	−	+	−	+/−	−	−

[a] Symbols +, a positive test; −, a negative test.

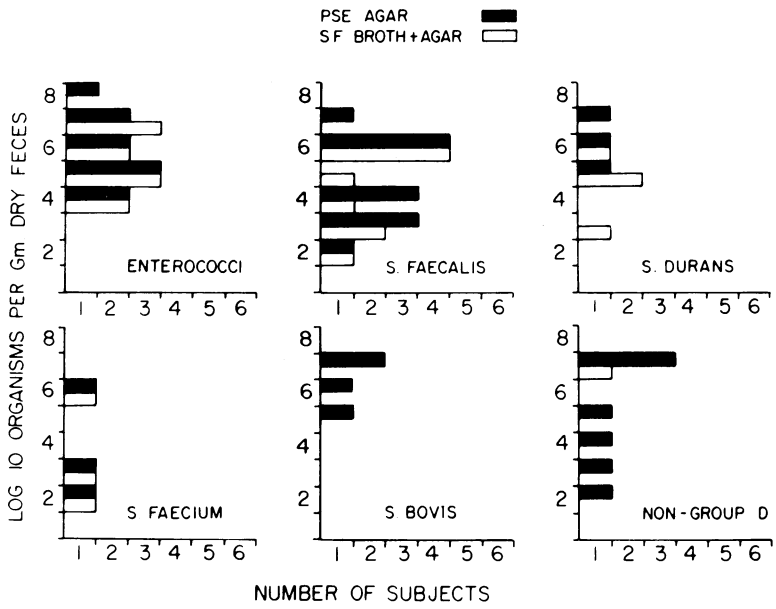

FIG. 2. *Quantitative recovery of streptococci from human feces on PSE agar and SF broth plus 1.5% agar.*

TABLE 2. *Recovery of presumptive group D streptococci and other cocci from human feces on various media*[a]

Bacteria	PSE agar	SF agar	SF broth
Enterococci, not speciated	10	10	9
S. faecalis	10	9	11
S faecalis var. *liquefaciens*	2	0	0
S. faecium	3	3	2
S. durans	3	4	1
S. bovis	4	0	0
Non-group D streptococci	7	1	0
Micrococcus or *Staphylococcus*	3	0	0

[a] Number of isolates, 25 subjects.

DISCUSSION

These results suggest that PSE agar is more suitable for recovery of presumptive group D streptococci than are SF media in quantitative bacteriological studies of feces. SF media are more specific for cultivation of enterococci than is PSE agar. Growth of test strains on PSE agar was not inhibited when compared with growth on blood-agar plates, in keeping with the conclusions of Isenberg et al. (4).

Characterization of isolates with the minimal screening tests indicated above does not define the strains as accurately as would the use of additional and more exacting taxonomic tests. Our purpose was to show the distribution of different presumptive group D streptococci which can be quantitatively recognized and isolated from overwhelming numbers of other bacteria in fecal material on PSE agar. The categorization of all catalase-positive gram-positive cocci as micrococci or staphylococci would eliminate catalase-positive streptococci from consideration, but such strains are rare. The lack of serological grouping of isolates allows for only presumptive identification of the esculin-hydrolyzing strains which grow on 40% bile agar and at 45 C as group D streptococci. Other streptococci, such as those in group Q and some strains of *S. mutans* and *S. sanguis*, would also give these reactions. None of the tests used in this study would have ruled out the possibility that some of the strains were group Q. In particular, some of the strains identified as *S. faecalis* may well have been group Q streptococci. This should be further investigated since Nowlan and Deibel (5) found group Q strains in 3 of 14 samples of human fecal material, whereas Isenberg et al. (4) reported none from the 25 samples he examined. All strains of *S. mutans* hydrolyze esculin and occasional strains will grow in the presence of 40% bile and at 45 C (1, 2). They do not grow in 6.5% NaCl and do ferment all four carbohydrates tested and might therefore be confused with *S. bovis*. Some strains of *S. sanguis* have similar characteristics with regard to esculin hydrolysis and growth in the presence of 40% bile, 6.5% NaCl, and at 45 C (1, 2). Their

fermentation of the four carbohydrates is the same as that of *S. durans*.

Among the small number of subjects reported here, specimens from 8% yielded no growth in SF broth; all specimens yielded presumptive group D streptococci on PSE agar. PSE agar also has the following advantages: (i) extraordinary conditions for incubation are not needed; (ii) distinct reactions appear rapidly (within 18 hr of incubation); and (iii) it selects out *S. bovis*, and probably *S. equinus* (4), as well as other presumptive group D streptococci.

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