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Gentamicin-Thallous-Carbonate Medium for Isolation of Fecal Streptococci from Foods†

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Gentamicin-thallous-carbonate (GTC) agar was formualted by Donnelly and Hartman (Appl. Environ. Microbiol. 35:576-581, 1978) to select for fecal streptococci in sewage and water samples. The present study was conducted to determine the usefulness of GTC agar for the enumeration of fecal streptococci in foods. Comparisons were made with KF streptococcal (KF), Pfizer selective enterococcus (PSE), and thallous acetate (TA) agars. Samples of ground beef, pork sausage, frozen broccoli, frozen fish, and ice cream were examined. Presumptive streptococcal counts obtained on GTC agar were significantly higher than those obtained on KF and PSE agars and were comparable to those obtained on TA agar. GTC was more sensitive than KF or PSE agars primarily because of the recovery of greater numbers of *Streptococcus bovis* and *Streptococcus equinus* strains. Percentages of confirmed fecal streptococci obtained on GTC, KF, PSE, and TA agars were 70, 95, 80, and 74, respectively. Differences between these percentages were not statistically significant, but they indicated that selectivity of GTC agar could be improved. Advantages of using GTC agar to isolate fecal streptococci from foods include a short incubation time (16 to 18 h) and large, distinct colonies that facilitate rapid enumeration and subsequent confirmation.

Group D streptococci are prevalent in the fecal material of humans and other animals (4, 13, 18, 23). Therefore, group D streptococci have assumed some importance as indicators of fecal contamination of foods and water. Caution and discretion must be exercised in attributing significance to the numbers and types of fecal streptococci present in foods, however, because of their ubiquitous distribution (15 and references cited therein). Nevertheless, counts of fecal streptococci often are helpful when determining the sanitary history of moderately heated, frozen, salted, or other foods and drinks in which coliforms might not have survived. Coliform-fecal streptococcus indices of water also can provide useful information (10).

Many media and methods are available for the enumeration of fecal streptococci (12), but each has one or more disadvantages. A new medium, gentamicin-thallous-carbonate (GTC) agar, was recently described by Donnelly and Hartman (6). GTC agar was superior to three commonly used media for the determination of fecal streptococci in feces and water. The purpose of this investigation was to extend these studies by evaluating the use of GTC agar for the enumeration of fecal streptococci in foods.

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MATERIALS AND METHODS

Selective media. GTC agar was prepared according to the procedure described by Donnelly and Hartman (6). KF streptococcal (KF) agar (BBL Microbiology Systems) and Pfizer selective enterococcus (PSE) agar (Pfizer, Inc.) were prepared according to the manufacturer's instructions. Thallous acetate (TA) agar was prepared as described by Barnes (1). The agar plates were dried overnight at 35°C and stored in vegetable crispers at 4°C to be used within a week.

Samples and sampling procedure. Five food types were examined: ground beef, pork sausage, frozen broccoli, frozen fish, and ice cream. Five samples of each type of food, each from a different production lot, were examined. All of the foods were purchased from local grocery stores except for two ground beef samples obtained from the Iowa State University Meat Laboratory. Frozen samples were thawed overnight at 4°C and tempered for 1 h at room temperature before they were examined; unfrozen samples were examined within 2 h of purchase.

A 50-g portion of a sample was aseptically weighed into a tared sterile Waring blender container. A 450-ml amount of sterile 0.1% peptone water was added, and the blender was operated at high speed for 1 to 2 min. Serial dilutions were made in 0.1% peptone, and 0.1 or 1.0 ml of appropriate dilutions was surface-plated in triplicate onto GTC, KF, PSE, and TA agar plates. Except where noted, the plates were incubated at 35°C for 24 to 48 h; colonies were counted, and the average plate count constituted the presumptive fecal streptococcus count. Ten colonies (or as many as available) from each medium were selected so that as many morphological types as possible were included.

The isolates were streaked onto modified Trypticase soy agar (BBL Microbiology Systems) plates (6) with the omission of 0.01% 2,3,5-triphenyltetrazolium chloride. The plates were incubated at 35°C for 24 h, colonies were selected, and the isolates were identified.

Identification media and procedures. Several isolated colonies were suspended in 3 ml of modified Minitek broth (BBL) (6). This cell suspension was used as inoculum for all of the tests, except the catalase test and the Gram stain; growth on the modified Trypticase soy agar plates was used for the latter two tests. Bile-esculin agar was a modification of Swan's (22) medium with the exclusion of horse serum. The 40% bile agar was made as described by Facklam (8), except that rabbit blood was excluded. Also described by Facklam (8) were the inulin and sorbose fermentation broths and the 6.5% NaCl broth (9). The pyruvate broth of Gross et al. (11) was used. Five percent sucrose agar plates were prepared as described by Niven et al. (16). To detect the presence of arginine dehydrolase, the medium and technique of Niven et al. (17) was used. Starch agar was that of Pavlova et al. (20), with the addition of 0.1% glucose (2). For the fermentation of arabinose, esculin, glycerol, lactose, mannitol, raffinose, sorbitol, and sucrose, the BBL Minitek identification system was used. To determine the reduction of tetrazolium, modified Trypticase soy agar plus 0.01% 2,3,5-triphenyltetrazolium chloride plates were used. All tests were incubated at 35°C and examined after 24 h, except for the NaCl, inulin, and sorbose tests, which were examined after 24, 48, and 72 h of incubation.

Figure 1 depicts the identification schema used to assign specific epithets to the isolates. With the exception of *Streptococcus uberis*, all species listed in Fig. 1 were considered as fecal streptococci. As noted by Deibel (5), reaction patterns were observed that deviated slightly from those shown in Fig. 1; in those instances, the overall results were evaluated before an isolate was named.

RESULTS

Growth was always evident first on GTC agar, usually after incubation for only 16 to 18 h. Colonies were not counted, however, until growth was evident on all of the other media. With the ice cream, frozen broccoli, and fish samples, 24-h incubation was necessary; with ground beef and pork sausage samples, 48-h incubation was required for typical colonies to develop on some of the media.

The data in Table 1 summarize the results on yields and overall selectivity of GTC, KF, PSE, and TA agars in isolating fecal streptococci from ground beef, pork sausage, frozen broccoli, frozen fish, and ice cream. The counts obtained from ground beef on GTC and TA agars were similar. Mean counts obtained on PSE agar were lower than those on GTC and TA agars, but these differences were not significant statistically (21). On all three media, however, significantly greater numbers of streptococci were recovered than on KF agar at a least significant difference (LSD) level of 0.01. *S. faecium* was the predominant species recovered on all four

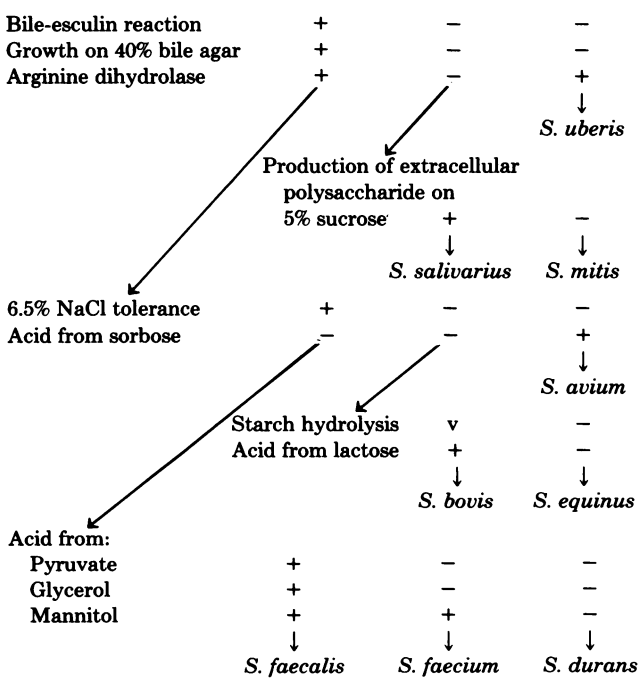

FIG. 1. *Flow chart for identification of fecal streptococci and S. uberis. v, Variable.*

TABLE 1. *Log geometric mean counts and percentage of fecal streptococci isolated from different foods*[a]

Food	Mean counts (%) of streptococci on given medium			
	GTC	KF	PSE	TA
Ground beef	5.24 (68)	3.02 (86)	4.98 (66)	5.32 (74)
Pork sausage	7.08 (88)	6.03 (93)	6.13 (78)	6.98 (72)
Frozen broccoli	5.99 (70)	2.92 (98)	4.11 (88)	5.94 (88)
Frozen fish	3.86 (96)	1.61 (100)	2.65 (92)	3.32 (98)
Ice cream	2.72 (30)	1.04 (96)	1.61 (78)	2.33 (40)
Overall means	4.98 (70)	2.84 (95)	3.90 (80)	4.78 (74)

[a] Each value represents the average results of experiments with five different lots of food.

media (Table 2); also, corynebacteria were recovered on GTC agar, staphylococci were recovered on PSE agar, and pseudomonads were recovered on TA agar.

A statistical analysis of the presumptive fecal streptococcal counts from pork sausage (Table 1) revealed that no significant differences were observed between the counts on GTC and TA agars or on PSE and TA agars. However, the counts obtained on GTC agar were significantly greater (LSD, 0.01) than those obtained on PSE agar, and counts on all three media were significantly higher (LSD, 0.01) than those on KF agar. Differences between the percentages of fecal streptococci isolated on all four media were not significant; *S. bovis* and *S. faecium* were the predominant species. As with the ground beef samples, staphylococci and pseudomonads also were recovered on PSE and TA agars, respectively.

Statistical analyses of the presumptive fecal streptococcal counts from frozen broccoli (Table 1) showed that counts on GTC and TA agars were significantly higher (LSD, 0.01) than those on PSE agar; all three counts were significantly higher (LSD, 0.01) than those on KF agar. Again, there were no significant differences between the percentages of fecal streptoccoci confirmed. Substantial numbers of *S. uberis* were among the nonfecal organisms isolated on GTC and TA agars (Table 2).

Results from the fish samples (Table 1) showed that the presumptive fecal streptococcal counts obtained on GTC, PSE, and TA agars were not significantly different, but the counts on all three media were significantly greater (GTC agar—LSD, 0.01; PSE and TA agars—LSD, 0.05) than those obtained on KF agar. In terms of selectivity, all four media yielded high percentages of fecal streptococci. Consequently, the overall differences were not statistically significant. More *S. bovis* strains were recovered on GTC and TA agars than on KF or PSE agars (Table 2).

The presumptive fecal streptococcal counts from ice cream samples obtained on GTC and TA agars (Table 1) were significantly greater (GTC agar—LSD, 0.01; TA agar—LSD, 0.05) than those obtained on PSE agar and also higher (LSD, 0.01) than those obtained on KF agar. Counts obtained on KF and PSE agars were not significantly different. In terms of selectivity, great differences existed between the percentages of confirmed fecal streptococci (Table 1). Despite the large apparent differences, they

TABLE 2. *Numbers of fecal streptococci, other streptococci, and other microorganisms isolated on four different media when five types of foods were plated*

Organisms	No. isolated on given medium			
	GTC	KF	PSE	TA
Fecal streptococci				
S. faecium	36	43	63	44
S. faecium (*S. casseliflavus*)	6	16	7	7
S. faecium (*S. durans*)	19	28	17	24
S. bovis (arginine dihydrolase negative)	34	20	35	25
S. bovis (arginine dihydrolase positive)	57	7	21	61
S. faecalis	13	71	29	13
S. avium	2	7	17	2
S. equinus	9	4	4	9
Other streptococci				
S. uberis	17	4	5	10
S. salivarius	7	0	0	3
S. cremoris-S. lactis	1	0	1	7
S. sanguis	7	0	0	0
Other microorganisms				
Staphylococcus	3	3	31	3
Corynebacterium	14	0	4	10
Lactobacillus	7	2	4	10
Pseudomonas	1	0	0	17
Bacillus	14	0	0	2
Shigella-like	1	1	0	0
Serratia	2	0	0	0
Citrobacter	1	0	0	0
Micrococcus	0	0	1	0
Yeast	0	0	0	1

were not statistically significant. This was caused by sample variance because five different flavors of ice cream were examined. Most of the bacteria isolated on GTC agar (Table 2) that were not fecal streptococci were bacilli, corynebacteria, lactobacilli, and *S. salivarius*; on TA agar, they were corynebacteria, lactobacilli, and *S. lactis*.

DISCUSSION

In overall efficiency, GTC agar recovered significantly greater numbers of presumptive fecal streptococci than did KF or PSE agar (Table 1). The efficiency of GTC agar paralleled that of TA agar. Both of these observations also were made with water samples (6). In terms of selectivity (Table 1), however, GTC agar was inferior to the other media. The highest overall percentages of confirmed fecal streptococci were 95% on KF agar, 80% on PSE agar, 74% on TA agar, and 70% on GTC agar. The differences between the percentages were not statistically significant. The low overall percentages of confirmation of colonies from GTC and TA agars were influenced substantially by the small numbers of fecal streptoccoci isolated from the ice cream samples. A major proportion of the ice cream isolates on GTC and TA agars were corynebacteria and lactic streptoccoci. On the basis of the 944 isolates identified, it seemed that, when ice cream and other samples (cottage cheese [unpublished data]) containing certain species of bacteria were examined, the selective agents in GTC and TA agars were inadequate to retard the growth of "background" bacteria for more than 24 h.

Even though the GTC agar formulation used in this study was not as selective as KF or PSE agar, GTC agar is a more sensitive medium because of the greater numbers of *S. bovis* and *S. equinus* strains that can form colonies on it. One hundred strains of *S. bovis* and *S. equinus* were isolated on GTC agar, whereas 31, 60, and 95 strains were isolated on KF, PSE, and TA agars, respectively. The number of strains isolated on GTC agar was significantly higher than those isolated on KF agar (LSD, 0.01) and PSE agar (LSD, 0.05), but was not significantly different from the number of strains isolated on TA agar. Therefore, the ability of GTC agar to allow the growth of larger numbers of *S. bovis* and *S. equinus* deems it a more sensitive medium than KF or PSE agar for recovery of the entire spectrum of fecal streptococci. This conclusion may be obscured by the manner in which we presented the data. Only 13 *S. faecalis* and 2 *S. avium* isolates (total, 15) were identified from GTC agar, for example (Table 2), whereas 71 *S. faecalis* and 7 *S. avium* isolates (total, 78) were identified from KF agar. Thus, GTC agar may seem less sensitive than KF agar for these two species. Counts of fecal streptococci on GTC agar were 14 times greater than counts on KF agar (Table 1). The recovery on GTC agar of vastly greater numbers of *S. bovis* and *S. equinus*, compared with recoveries on KF agar, resulted in proportional decreases of percent recoveries of fecal streptococci that initiated growth on both media at about the same frequencies.

GTC agar possesses several other advantages as a selective medium for fecal streptococci. It was easy to differentiate the fecal streptococcal colonies from the other colonies by the presence of dark halos and by the colonial morphology. The colonies on GTC agar were larger than those on KF, PSE, and TA agars (T. S. Thian, M.S. thesis, Iowa State University, Ames, 1978); this observation was also reported by Donnelly and Hartman (6). In addition, growth in GTC agar was observed after 16 to 18 h; almost always the colonies were typical of fecal streptococci. Thus, the percentage confirmations reported herein were grossly exaggerated in disfavor of GTC agar. In another study (G. A. Thibodeau, M.S. thesis, Iowa State University, Ames, 1978), using clinical specimens, growth on GTC agar also was evident after 18 h of incubation. Despite reports that PSE agar, because of the short (24-h) incubation time, was the favored selective medium for group D streptococci (3, 19), GTC agar surpassed it with a shorter incubation time. Efthymiou et al. (7) provided evidence that a short incubation time of 16 to 17 h was successful in isolating enterococci from cheese, and the authors believed that this should also be applicable for group D streptococci. If, by applying an incubation time of 16 to 18 h, the selectivity of modified GTC agar increased still further, GTC agar definitely would be the ideal medium to use to isolate fecal streptococci from foods, water, or other sources.

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