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Comparison of Fluorescent Gentamicin-Thallous-Carbonate and KF Streptococcal Agars To Enumerate Enterococci and Fecal Streptococci in Meats†

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Two selective and differential media were compared for their abilities to enumerate enterococci and fecal streptococci in pork, beef, and poultry products. Counts obtained on KF streptococcal (KF) agar were compared with counts obtained on fluorescent gentamicin-thallous-carbonate (fGTC) agar. Reactions of 13 known enterococcal species were also observed. All 13 species of enterococci as well as *Streptococcus bovis* and *Streptococcus equinus* grew equally well on fGTC agar. KF streptococcal medium allowed growth of most species of enterococci but not *S. bovis* and *S. equinus*. Quantitative comparisons between the two media inoculated with pure cultures of known species of enterococci revealed equivalent plate counts following incubation. However, when meat samples were plated, counts on fGTC agar were consistently and significantly higher than counts on KF agar for all sample sources.

Fecal streptococci, including enterococci, have been considered an alternative potential indicator of fecal pollution of foods because they have an advantage over coliforms, the primary fecal indicator, in that they are more resistant than coliforms to most environmental insults. This trait is shared by many potential pathogens; therefore, enterococci can help determine the sanitary history of moderately heated, frozen, dried, or salted foods and other foods in which coliforms might not have survived (13). Enterococci are also important in human infections such as endocarditis and bacteremia, and they may be resistant to clinically important antibiotics (9).

Many media have been developed to isolate and enumerate enterococci (11). In 1961, Kenner et al. (3) described new solid and liquid media (KF streptococcal broth and agar) for enumerating enterococci. These media contained, among other ingredients, sodium azide, bromcresol purple, and 2,3,5-triphenyltetrazolium chloride. A new medium, gentamicin-thallous-carbonate (GTC) agar, was reported in 1978 (1). It was superior to KF agar for the enumeration of fecal streptococci in fecal and surface-water samples. GTC agar was modified (fluorescent GTC [fGTC] agar) by Littel and Hartman (8) in 1983 to make it more differential by incorporating a colorimetric starch substrate plus a fluorogenic substrate, allowing differentiation of colonies on the agar surface. Gentamicin and thallous acetate were the major selective agents. $NaHCO_3$, Tween 80, and KH_2PO_4 were added as specified earlier by Lachica and Hartman (7) to stimulate the growth of group D streptococci. The incorporation of amylose azure and 4-methylumbelliferyl-α-D-galactoside allowed differentiation of the fecal streptococci into three phenotypic groups: starch hydrolysis and fluorescence, no starch hydrolysis but fluorescence, and no starch hydrolysis or fluorescence (8).

With the recent changes in classification of this group of organisms, questions about the selectivity of the media for new members of the genus *Enterococcus* have arisen. The abilities of these media to recover all species of enterococci and fecal streptococci, as well as the differentiating characteristics of each species on both media, are the subject of this study.

Cultures. A total of 59 strains representing 13 species of enterococci, *Streptococcus bovis*, and *Streptococcus equinus* were collected from various sources (5). Cultures also were isolated from pork carcasses during slaughter and from fresh and spoiled pork sausage, poultry, and beef products. All cultures were isolated as described by Knudtson and Hartman (6).

Media. KF streptococcal agar (KF; Difco Laboratories, Detroit, Mich.) was prepared according to the manufacturer's instructions. Portions (1 ml each) of appropriate dilutions were used for KF agar pour plates. Duplicate plates were incubated at 37°C for 48 h. Colonies exhibiting a red or pink color were counted as streptococci (3). The second medium, fGTC agar (8), was prepared as specified by the authors; 0.1-ml portions of appropriate dilutions were plated in duplicate, and the plates were incubated at 37°C for 24 h. All except pinpoint colonies were counted as enterococci. Positive fluorescence and starch hydrolysis (zones of clearing) also were recorded.

Species identification. After the colonies on KF and fGTC plates had been counted, three colonies of each colony type on each medium were streaked for isolation on brain heart infusion agar (Difco). After 24 h of incubation, a Gram stain and catalase test were performed to verify gram-positive, catalase-positive colonies. The cultures also were tested on bile esculin agar (Difco) for their abilities to grow in bile and hydrolyze esculin. Cultures positive for these tests were identified to species level by using the classification schema developed by Knudtson and Hartman (5). This schema was developed by using API Rapid Strep and MicroScan Pos ID panels. The identities of the isolates were compared with results for known strains on both media.

In every instance, enterococcus counts made on pork, beef, and poultry samples by using fGTC agar were significantly higher ($P < 0.01$) than counts on KF agar (Fig. 1). One

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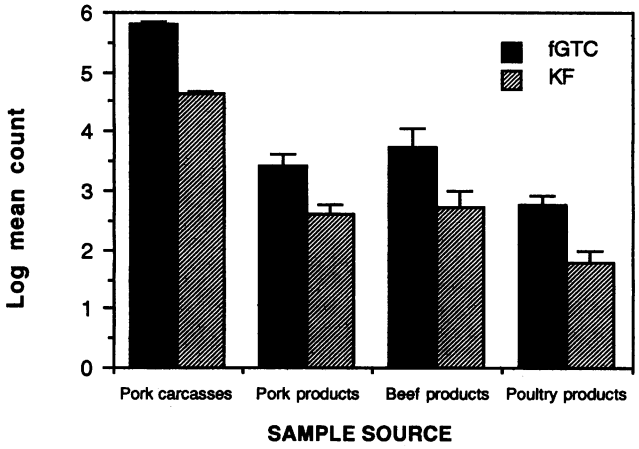

FIG. 1. Comparison of mean log counts on fGTC and KF agars of four sample sources (per 100 cm^2 for pork carcasses; per gram for other products). All of the differences between values obtained with the two media were significant ($P < 0.01$).

explanation is that a larger proportion of injured enterococci grew on fGTC agar than on KF agar. In 1961, Kenner et al. (3) stated that KF agar allowed the growth of *S. bovis*, *S. equinus*, and the enterococcal group consisting of *Streptococcus faecalis*, its varieties *Streptococcus faecalis* var. *liquifaciens* and *Streptococcus faecalis* var. *zymogenes*, and related *S. faecalis* biotypes. Significant classification changes have occurred since that time, and many newly classified species of enterococci may not have been tested for growth on KF agar. fGTC agar allows growth of *S. faecalis*, *Streptococcus faecium*, *S. bovis*, *S. equinus*, and *Streptococcus avium* (8). This medium was developed before the classification changes that began in 1984 (12). Newly classified species of enterococci had not been tested on this medium either.

When known species of enterococci were plated on each medium (Table 1), fGTC agar allowed growth of all enterococci and fecal streptococci tested. Strains on fGTC agar were differentiated into three groups according to the ability to fluoresce and hydrolyze starch. *Enterococcus faecalis*, *Enterococcus pseudoavium*, *Enterococcus solitarius*, and *S. equinus* made up a group that was both fluorescence and starch negative. *S. bovis* was positive for both fluorescence and starch hydrolysis. Most of the rest of the enterococcal species tested were fluorescence positive and starch negative. When *E. avium* and *Enterococcus faecium* were tested, only 38% and 71% of isolates produced fluorescence, respectively. Littel and Hartman (8) reported similar findings; when 87 strains of *S. faecium* were plated on fGTC agar, 80 (92%) showed fluorescence and 7 (8%) did not. KF agar did not allow growth of known strains of *Enterococcus cecorum*, *S. bovis*, or *S. equinus* (Table 1), but the rest of the enterococci grew on this medium. This medium contains sodium azide as a selective agent. Some streptococci, such as *S. bovis*, cannot initiate growth on media that contain azide (1). Most species reduced tetrazolium to some degree, and *E. faecalis* reduced it the most strongly (Table 1). Differences in the intensity of tetrazolium reduction were difficult to visualize and could be determined accurately only when plates containing different species were compared side by side. When counts obtained from plating diluted samples of known strains were compared (data not shown), there were no significant differences in numbers on the two media (except for the three species that did not grow on KF agar).

To determine whether differences in counts obtained from meat samples plated on the two media were a result of the recovery of species on fGTC agar that did not grow on KF agar, the identities of 175 isolates collected from pork carcasses were tabulated (Table 2). The distribution of most species of enterococci was similar on both media. No *E. cecorum*, *S. bovis*, or *S. equinus* was isolated from the pork carcasses by using either medium. Only *Enterococcus durans* and *Enterococcus casseliflavus* were isolated on fGTC agar and not on KF agar. These accounted for only very small percentages of isolates and could not be sufficient to account for the differences in counts.

Another possible explanation for higher recoveries on fGTC agar than on KF agar might be that organisms other than enterococci and fecal streptococci grew on fGTC agar, giving false-positive results. However, more than 95% of isolates from the colony types counted as enterococci on fGTC agar were identified as enterococci or fecal streptococci (pinpoint colonies were not counted, although some

TABLE 1. Growth characteristics of known species of enterococci and fecal streptococci on fGTC and KF agars

Species	No. of strains	% Positive on fGTC agar[a]			KF agar	
		Growth	F	S	% Positive for growth	Colony color
E. avium	8	100	38	0	100	Pink
E. casseliflavus	5	100	100	0	100	Pink
E. cecorum	2	100	100	0	0	
E. durans	4	100	100	0	100	Pink
E. faecalis	8	100	0	0	100	Dark red
E. faecium	7	100	71	0	100	Pink
E. gallinarum	4	100	100	0	100	Red
E. hirae	6	100	100	0	100	Pink
E. malodoratus	2	100	100	0	100	Red
E. mundtii	4	100	100	0	100	Red
E. pseudoavium	2	100	0	0	100	Red
E. raffinosus	1	100	100	0	100	Red
E. solitarius	1	100	0	0	100	Red
S. bovis	4	100	100	100	0	
S. equinus	1	100	0	0	0	

[a] F, fluorescence; S, starch hydrolysis.

TABLE 2. Species isolated from pork carcasses on fGTC and KF agars

Species	fGTC agar		KF agar	
	No. of isolates	% of total	No. of isolates	% of total
E. faecalis	49	69	87	84
E. faecium	15	19	7	6
E. durans	2	3	0	0
E. casseliflavus	1	2	0	0
E. malodoratus	1	2	2	2
E. solitarius	1	2	2	2
E. pseudoavium	1	2	4	4
Unidentified *Enterococcus* sp.	1	2	2	2
Total	71	100	104	100

were identified to be certain that they were not enterococci) (4). These results were similar to those reported by Littel and Hartman (8), who found that 90% of isolates from sewage and fecal samples were enterococci or fecal streptococci. Some care must be exercised, however, when either KF or fGTC agar is used. A heavy background of *Lactobacillus* colonies appeared on plates from samples highly contaminated with lactobacilli (such as pork sausage whose shelf-life has expired), and *Enterococcus* colonies became atypical (6).

Hartman et al. (2) stated that many, if not most, of the microorganisms in certain foods and natural environments may be in a physiological state different from that of similar strains cultivated in the laboratory. With laboratory stock cultures used as test material, continuation of bacterial growth is the concern; with bacteria from the natural environment, however, the problem seems to be not only growth but also growth initiation (10). The overall effect is that a medium will usually be more inhibitory to bacteria from natural products than to rapidly growing laboratory cultures. If this is so, then it is possible that the differences between counts made on fGTC and KF agars could be a result of the inhibitory properties of KF agar (which contains sodium azide) to enterococci and fecal streptococci present in natural products.

In conclusion, fGTC agar is a selective and differential medium for enterococci and fecal streptococci. It is as good as, if not better than, KF agar for enumerating enterococci and fecal streptococci in foods. Furthermore, fGTC agar enables colony differentiation that might be useful in some circumstances. However, fGTC agar can yield falsely high counts when excessive numbers of lactobacilli are present because enterococcal colonies become atypical and cannot be differentiated from whitish, pinpoint *Lactobacillus* colonies on crowded plates.

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