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Suitability of Selective Plating Media for Recovering Heat- or Freeze-Stressed *Escherichia coli* O157:H7 from Tryptic Soy Broth and Ground Beef

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The efficacy of tryptic soy agar (TSA), modified sorbitol MacConkey agar (MSMA), modified eosin methylene blue (MEMB) agar, and modified SD-39 (MSD) agar in recovering a five-strain mixture of enterohemorrhagic *Escherichia coli* O157:H7 and five non-O157 strains of *E. coli* heated in tryptic soy broth at 52, 54, or 56°C for 10, 20, and 30 min was determined. Nonselective TSA supported the highest recovery of heated cells. Significantly ($P \leq 0.05$) lower recovery of heat-stressed cells was observed on MSMA than on TSA, MEMB agar, or MSD agar. The suitability of MEMB agar or MSD agar for recovery of *E. coli* O157:H7 from heated or frozen (−20°C) low- or high-fat ground beef was determined. Recovery of *E. coli* O157:H7 from heated ground beef was significantly ($P \leq 0.05$) higher on TSA than on MEMB agar, which in turn supported higher recovery than MSD agar did; MSMA was inferior. Recovery from frozen ground beef was also higher on MEMB and MSD agars than on MSMA. Higher populations were generally recovered from high-fat beef than from low-fat beef, but the relative performance of the recovery media was the same. The inability of MSMA to recover stressed cells of *E. coli* O157:H7 underscores the need to develop a better selective medium for enumerating *E. coli* O157:H7.

Physiologically deficient or injured bacterial cells can result from exposure to heat, freezing, desiccation, or chemicals. Injury may be manifested by a loss of cellular membrane integrity, increased sensitivity to sodium chloride or bile salts, and/or degradation of rRNA (19). Injured cells of pathogenic bacteria may undergo repair and regain the ability to cause illness when conditions are favorable for growth. However, selective media used to detect microorganisms in foods and environmental samples often do not support the repair of injured cells. Selective agents, such as bile salts and crystal violet, may inhibit repair of injured cells (13). As a consequence, injured bacterial cells may not be detected during routine laboratory analyses.

Consumption of undercooked ground beef containing *Escherichia coli* O157:H7 has resulted in illness ranging from mild bloody diarrhea to severe and life-threatening hemolytic-uremic syndrome (2). Direct-plating media for detecting healthy as well as all injured cells of *E. coli* O157:H7 in raw and processed foods have yet to be developed, although methods for accurate biochemical and immunological characterization of *E. coli* O157:H7 isolated from foods have been described (6, 10, 17). Identification methods rely, in part, on the inability of *E. coli* O157:H7 to ferment sorbitol and produce β-glucuronidase and to express specific antigenic determinants detectable by immunoblotting techniques (15). While DNA probe and verotoxin assays facilitate rapid detection and identification, many laboratories lack the capability of carrying out such techniques. Diagnosis is more often dependent upon isolation of *E. coli* O157:H7 cells followed by biochemical characterization.

For the detection of injured cells, resuscitation in an enrichment broth is often required. The time required for repair is critical, because multiplication of other microorganisms may occur before the injured cells of the target organism can recover. Several plating media have been shown to be adequate for selective enumeration of *E. coli* O157:H7, but sorbitol MacConkey agar (SMA) supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG) (MSMA) has been recommended (9). Abdul-Raouf et al. (1), however, reported that MSMA failed to detect heat-stressed *E. coli* O157:H7 cells in roasted beef. Conner and Hall (5) reported that MSMA was inadequate for recovering *E. coli* O157:H7 cells from frozen chicken meat. These observations have raised concern over the suitability of MSMA for routine enumeration of *E. coli* O157:H7 in foods. This study was undertaken to evaluate modified eosin methylene blue (MEMB) and modified SD-39 (MSD) agars for their suitability for the recovery of *E. coli* O157:H7 cells injured by being heated in tryptic soy broth (TSB) and to determine their efficacy for enumerating heat- and freeze-stressed *E. coli* O157:H7 cells in low- and high-fat ground beef.

MATERIALS AND METHODS

Inoculum preparation. Five enterohemorrhagic *E. coli* O157:H7 strains were studied: strains E0019, 505B, and CA-1 (beef isolates), strain 932 (human isolate), and strain 204P (pork isolate). Five non-O157 (nonenterohemorrhagic) strains of *E. coli* (two O6:NM strains [strains SSU8470 and F536c2], two O6:H17 strains [strains F526 and EDL 737], and strain JM109) were also investigated. All *E. coli* O6 strains were provided by Nancy Puhr, Centers for Disease Control and Prevention, Atlanta, Ga.; JM109 was a laboratory stock strain. Cultures were maintained in tryptic soy agar (TSA; pH 7.3) (Difco, Detroit, Mich.) slants at 5°C and activated by transferring loop inocula into 10 ml of TSB (Difco) at 37°C. Three successive 24-h culture transfers in 16- by 150-mm screw-cap test tubes were made in triplicate before use as inocula for experiments.

Preparation of media. Modified Levine's eosin methylene blue agar (MEMB; pH 6.8 ± 0.2) was prepared by adding 10 g of sorbitol (Sigma Chemical Co., St. Louis, Mo.), 5 g of NaCl (J. T. Baker, Inc., Phillipsburg, N.J.), and 0.02 g of sodium novobiocin (Sigma) to 1 liter of Levine's eosin methylene blue agar without lactose (BBL, Becton Dickinson Micro System, Cockeysville, Md.). All

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ingredients were mixed, brought to a boil, and autoclaved at 121°C for 15 min to sterilize.

The MSD agar formula is a modification of SD-39 agar manufactured by QA Life Sciences Inc., San Diego, Calif. (12). Modified SD-39 (MSD; pH 7.3 ± 0.2) agar contained (per liter) 5 g of Proteose Peptone (Difco), 3 g of yeast extract (Difco), 5 g of NaCl (J. T. Baker), 10 g of L-lysine monohydrochloride (Sigma), 2.5 g of Bacto-dextrose (Difco), 1.5 g of magnesium sulfate pentahydrate ($MgSO_4 \cdot 7H_2O$; Merck and Co., Rahway, N.J.), 0.038 g of monensin (Sigma), 0.5 g of sodium glucuronate (Sigma), 0.0075 g of novobiocin (Sigma), 0.12 g of phenol red (Sigma), 0.01 g of MUG (Oxoid-Unipath, Ogdensburg, N.Y.), and 15 g of Bacto Agar (Difco). All ingredients were mixed, brought to a boil, and heated at 121°C for 15 min.

TSA (pH 7.0; Difco) was prepared as specified by the manufacturer. MSMA (pH 6.9) was prepared by adding MUG (0.1 g $liter^{-1}$) to SMA (Oxoid-Unipath) before heat sterilization.

All media were tempered to 50°C before being dispensed into 100- by 15-mm plates. Media were prepared and poured into petri plates 3 to 4 days before use and held at 5°C between the time of preparation and use.

Heating studies using broth. Equal volumes of 24-h cultures from each of the five *E. coli* O157:H7 (enterohemorrhagic) strains were aseptically combined and thoroughly mixed. A mixture of the five non-O157:H7 (nonenterohemorrhagic) strains was likewise prepared. Cell suspensions (ca. 10^7 CFU ml^{-1}), prepared by diluting the five-strain cocktail mix 100-fold in sterile 0.1% peptone water (Difco), were used as inocula. Erlenmeyer flasks (250 ml) containing 100 ml of sterile TSB (pH 7.3) were tempered at 52, 54, or 56°C in an Aquatherm water bath shaker (New Brunswick Scientific, New Brunswick, N.J.) with constant agitation (100 rpm) before addition of inoculum (1 ml). The water level in the bath was adjusted such that the level of TSB was well below the level of the water surface during heat treatment. Samples (1 ml) were withdrawn after being heated for 0, 10, 20, or 30 min. Serial dilutions (1:10) in sterile 0.1% peptone water were prepared, and appropriate dilutions were surface plated in duplicate (0.1 ml) or quadruplicate (0.25 ml) on TSA, MSMA, MEMB agar, and MSD agar. Colonies formed after incubation at 37°C for 48 h were enumerated. Each experiment was conducted in triplicate.

A second series of experiments was carried out to study thermal inactivation characteristics of individual strains. Five *E. coli* O157:H7 strains and five non-O157:H7 strains were subjected to the same experimental protocol described for mixtures of strains, except that inocula were prepared by diluting 24-h cultures 100-fold with sterile 0.1% peptone water. Heating studies were conducted only at 54°C, and heating times were 15 and 30 min. Initial populations before heat treatment were determined by plating on TSA.

Heating studies with ground beef. The ability of MEMB and MSD agars to support resuscitation and colony formation by five heat-injured strains of *E. coli* O157:H7 individually inoculated into low- and high-fat ground beef was determined. Low-fat (13.2%) and high-fat (28.4%) ground beef samples were prepared by grinding top sirloin and round chuck beef, respectively, in an electric food grinder equipped with a plate with 0.5-cm-diameter holes (model 2250; Rival Manufacturing Co., Kansas City, Mo.). Ground beef containing each level of fat was thoroughly mixed by hand and subdivided into three equal portions (designated replicates 1, 2, and 3). Five 100-g quantities of ground beef from each replicate were weighed, deposited in labeled Ziploc bags, sealed, and stored at −20°C until used.

Samples were thawed at 1°C for 16 h before being inoculated with individual test strains. Each low- and high-fat sample (100 g) was inoculated with 1 ml of a cell suspension (ca. 10^7 CFU ml^{-1}), prepared by diluting a 24-h culture in sterile 0.1% peptone water. Inoculated ground beef was thoroughly mixed by hand for 2 min, and three 10-g portions were placed in labeled polyethylene plastic bags. The ground beef was spread evenly (0.3 cm thick by 8 cm wide) in bags to facilitate uniform heat transfer during heat treatment. Six samples of each replicate of beef containing each level of fat were individually sealed in bags with a Thermal Impulse Sealer (Vertrod, Brooklyn, N.Y.). Care was taken to minimize the presence of air. Four samples (two high fat and two low fat) were simultaneously placed in an Aquatherm water bath shaker maintained at 56°C. The samples were kept submerged (2.5 cm from the water surface and 7.6 cm apart) in a vertical position throughout the heat treatment for 15 or 30 min. Unheated samples (0-min heating) served as controls. Each sample was dipped for 30 s in chilled (ca. 5°C) water containing 300 μg of sodium hypochlorite ml^{-1} (Aldrich Chemical Co., Inc., Milwaukee, Wis.), rinsed in sterile deionized water for 30 s, and aseptically opened with a surgical knife. The sample was then placed in a stomacher bag with 40 ml of sterile 0.1% peptone water and pummeled for 1 min. Appropriate serial dilutions (1:10) of slurry made in sterile 0.1% peptone water were surface plated in duplicate (0.1 ml) or quadruplicate (0.25 ml) on TSA, MSMA, MEMB agar, and MSD agar. All plates were incubated at 37°C for 48 h before colonies were counted. At least five presumptive colonies per sample were picked and confirmed by microscopic examination, biochemical testing with miniaturized API 20E diagnostic kits, and the *E. coli* O157 latex agglutination assay. Heating studies were conducted in triplicate.

Freezing studies with ground beef. Ground (9/64-in. [0.36-cm] grind) top sirloin and round chuck beef containing 14.2% (low) and 30.9% (high) fat, respectively, were purchased at a local supermarket. Five *E. coli* O157:H7 strains (E0019, 505B, CA-1, 932, and 204P) were individually tested. Cell suspensions (ca. 10^7 CFU ml^{-1}) prepared by diluting 24-h cultures 100-fold in sterile 0.1% peptone water were used as inocula. Five samples (ca. 15°C) each of low- and high-fat ground beef (150 g) were individually inoculated with individual test strains (1.5 ml of cell suspension per 150 g of ground beef). Inoculated beef was hand mixed for 2 min and divided into three 50-g portions. Two portions were individually sealed in labeled polyethylene Ziploc bags and stored at −17 ± 1°C for 1 or 14 days. The remaining 50-g portion served as the control (day 0, unfrozen).

Samples were thawed at room temperature (23 to 24°C) for 1 h before being subjected to analysis for populations of *E. coli* O157:H7. Subsamples (10 g) were combined with 40 ml of sterile 0.1% peptone water and pummeled for 1 min. Serial dilutions were prepared in sterile 0.1% peptone water, and samples were plated in duplicate (0.1 ml) or quadruplicate (0.25 ml) on TSA, MSMA, MEMB agar, and MSD agar. Plates were incubated at 37°C for 48 h before colonies were counted, and presumptive colonies were picked (at least five isolates per sample) and confirmed by microscopic examination, biochemical testing with miniaturized API 20E diagnostic kits, and the *E. coli* latex agglutination assay. Portions (40 g) of beef not analyzed after 14 days of storage at −17 ± 1°C were returned to the freezer and analyzed after an additional 46 days of storage.

Statistical analysis. A split-plot experimental design was used for both heating and freezing studies. All data were analyzed by using the general linear model of the Statistical Analysis Systems procedures (16). Mean separation was obtained by the least-squares difference method.

RESULTS AND DISCUSSION

Although the frequency of outbreaks of food-borne illness epidemiologically linked to foods containing *E. coli* O157:H7 has increased during the last decade, the efficacy of detection and isolation methods is still inadequate. For selective enumeration, SMA supplemented with MUG (MSMA) is the recommended medium. Conner and Hall (5) and Abdul-Raouf et al. (1), however, noted that MSMA failed to resuscitate a portion of the viable cells of *E. coli* O157:H7 in frozen chicken and heated roasted beef, respectively. MSMA and two other selective media formulated in our laboratory were therefore evaluated for the ability to recover heat- or freeze-stressed *E. coli* O157:H7 from TSB and ground beef.

Survival curves for a five-strain mixture of *E. coli* O157:H7 cells heated in TSB at 52, 54, or 56°C for 10, 20, and 30 min and subsequently plated on TSA, MSMA, MEMB agar, or MSD agar are shown in Fig. 1a. Changes in the number of viable *E. coli* O157:H7 cells heated at 52°C for 30 min were insignificant as evidenced by recovery on MEMB agar, MSD agar, and nonselective TSA. However, when MSMA was used as the plating medium, the number of colonies formed by *E. coli* O157:H7 cells heated at 52°C for 30 min was significantly smaller ($P \leq 0.05$) than those on TSA, MEMB agar, and MSD agar, indicating that a portion of heat-injured cells failed to resuscitate and form colonies on MSMA. At 54°C, decreases in populations recovered on all four media were observed as heating progressed. No significant differences ($P \leq 0.05$) were observed when cells heated for 10 min were plated on TSA, MEMB agar, or MSD agar, but better recovery of cells heated for 20 or 30 min was observed on TSA than on MEMB agar or MSD agar. Regardless of the heating time at 54°C, recovery of viable cells on MSMA was significantly lower than recovery on TSA, MEMB agar, and MSD agar. The difference in the number of cells recovered became more apparent as the heating time was increased to 30 min, when the population of viable cells detected on TSA exceeded that of those on MSMA by approximately 10^3 CFU ml^{-1}. At 56°C, no viable cells were detected in 1 ml of a heated (30 min) cell suspension plated on MSMA. The number of viable cells recovered on MEMB and MSD agars was significantly larger ($P \leq 0.05$) than on MSMA but smaller than on TSA. No differences were observed in the performance of MEMB and MSD agars.

The ability of MSMA, MEMB agar, and MSD agar compared with nonselective TSA for recovering heated cells from a five-strain mixture of non-O157:H7 strains was also investigated (Fig. 1b). Treatment of non-O157:H7 strains at 52°C for

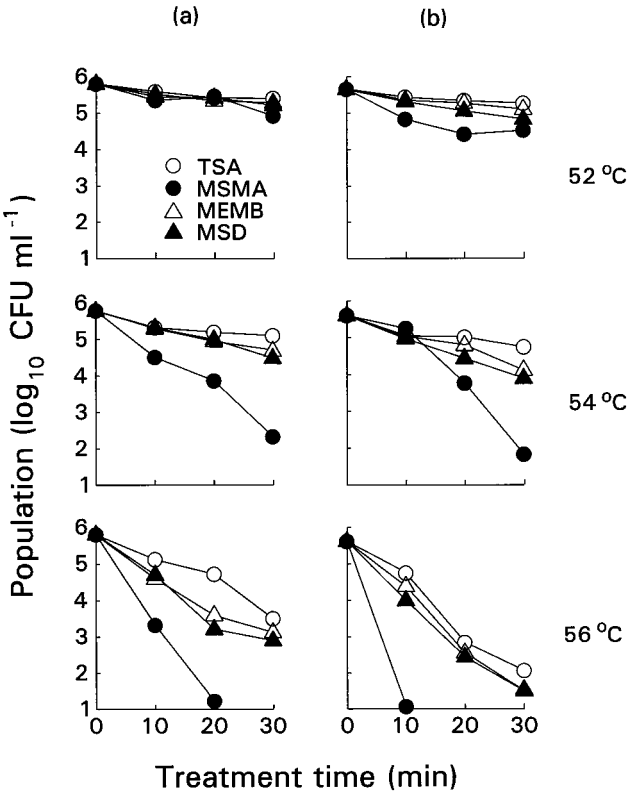

FIG. 1. Survival of a five-strain mixture of *E. coli* O157:H7 cells (a) and *E. coli* non-O157:H7 cells (b) heated in TSB at 52, 54, or 56°C for 10, 20, and 30 min and plated on TSA, MSMA, MEMB agar, and MSD agar.

30 min resulted in slight decreases in populations recovered on all four media. Regardless of the heating time at 52°C, TSA gave the highest recovery of non-O157 cells. Significantly smaller numbers ($P \leq 0.05$) of viable cells were recovered on MEMB and MSD agars than on TSA when cells were heated for 20 and 30 min. Poorest recovery was observed on MSMA for all heating times. Populations of heated (54°C) cells recovered on MSMA decreased significantly ($P \leq 0.05$) with each heating time. Differences were magnified when cells were heated at 56°C; only 19% of the viable cells heated at 56°C for 10 min were recovered on MSMA. Regardless of the heating temperature or time, larger numbers of viable *E. coli* O157:H7 cells than of non-O157:H7 cells were recovered on all respective plating media.

The influence of heating temperature and duration of treatment on the survival and recovery of cells of individual strains of *E. coli* O157:H7 and non-O157:H7 was determined. Table 1 shows populations ($\log_{10}$ CFU per milliliter) of all test strains of *E. coli* heated in TSB at 54°C for 15 and 30 min that were recovered on various plating media. Initial populations ranged from $10^{4.18}$ (*E. coli* JM109) to $10^{5.04}$ (*E. coli* O157:H7 strain CA-1) CFU ml^{-1}. Reductions in population occurred within 15 min and between 15 and 30 min. Non-O157:H7 *E. coli* strains were more sensitive to heat than were *E. coli* O157:H7 strains, confirming our observations on five-strain mixtures (Fig. 1). Heat treatment of all *E. coli* O157:H7 and non-O157:H7 strains for 15 min resulted in a significantly smaller number of viable cells recovered on MSMA than on TSA. The inability of MSMA to recover injured cells was more pronounced when cells were heated for 30 min. The number of viable cells from 6 of the 10 strains that formed colonies on MEMB and MSD agars was significantly smaller than the number detected on TSA, but both media were superior to MSMA for supporting colony formation by 9 strains. None of the strains used in this study produced sorbitol-positive colonies on MSMA within 48 h of incubation. Results also showed that strains vary in their sensitivity to heat. *E. coli* O157:H7 strain CA-1 appears to be the most heat resistant of the O157:H7 strains, while strain 505B appears to be the most sensitive. Roth et al. (14) also observed differences in recovery of heat-injured *E. coli* strains on selective media. Extreme caution must therefore be exercised in interpreting results of studies with stress-injured organisms, especially when only one strain is being evaluated and selective media are used for enumeration.

The suitability of MEMB and MSD agars compared with MSMA for supporting recovery of *E. coli* O157:H7 cells from

TABLE 1. Populations of *E. coli* O157:H7 and non-O157:H7 strains heated in TSB at 54°C for 15 or 30 min that were recovered on various plating media

Strain	Inoculum ($\log_{10}$ CFU ml^{-1})	Population ($\log_{10}$ CFU ml^{-1}) of strains after heat treatment for[a]:							
		15 min				30 min			
		TSA	MSMA	MEMB agar	MSD agar	TSA	MSMA	MEMB agar	MSD agar
O157:H7									
CA-1	5.04	4.85 a	4.44 c	4.54 bc	4.73 ab	4.71 a	2.97 b	4.50 a	4.47 a
932	4.67	4.46 a	4.07 c	4.26 b	4.25 b	4.24 a	2.97 c	4.08 a	3.68 b
505B	4.76	2.48 a	2.00 b	2.19 ab	2.18 ab	—[b]	—[c]	—[d]	—[d]
E0019	5.00	3.89 a	2.98 b	4.00 a	4.04 a	3.03 a	1.21 c	2.64 b	2.62 b
204P	5.04	4.69 a	3.76 c	4.50 b	4.66 a	4.33 a	2.65 c	3.99 b	3.97 b
Non-O157:H7									
F526	4.81	4.66 a	3.97 c	4.57 a	4.23 ab	4.10 a	2.51 c	3.65 b	3.72 b
F536c2	4.83	4.28 a	2.59 c	4.22 a	3.30 b	3.65 a	2.06 c	3.44 ab	3.30 b
EDL 737	4.72	4.38 a	3.47 b	4.35 a	4.35 a	4.14 a	2.40 d	3.79 b	3.28 c
SSU8470	4.87	4.57 a	3.68 c	4.40 b	4.53 a	4.10 a	2.38 d	3.51 c	3.77 b
JM109	4.18	4.15 a	2.84 c	3.88 b	3.92 b	3.54 a	1.56 d	3.82 c	3.15 b

[a] Values in the same row within the same treatment (15 or 30 min at 54°C) that are not followed by the same lowercase roman letter are significantly different ($P \leq 0.05$).
[b] Less than 100 CFU ml^{-1}.
[c] Less than 1 CFU ml^{-1}.
[d] Less than 10 CFU ml^{-1}.

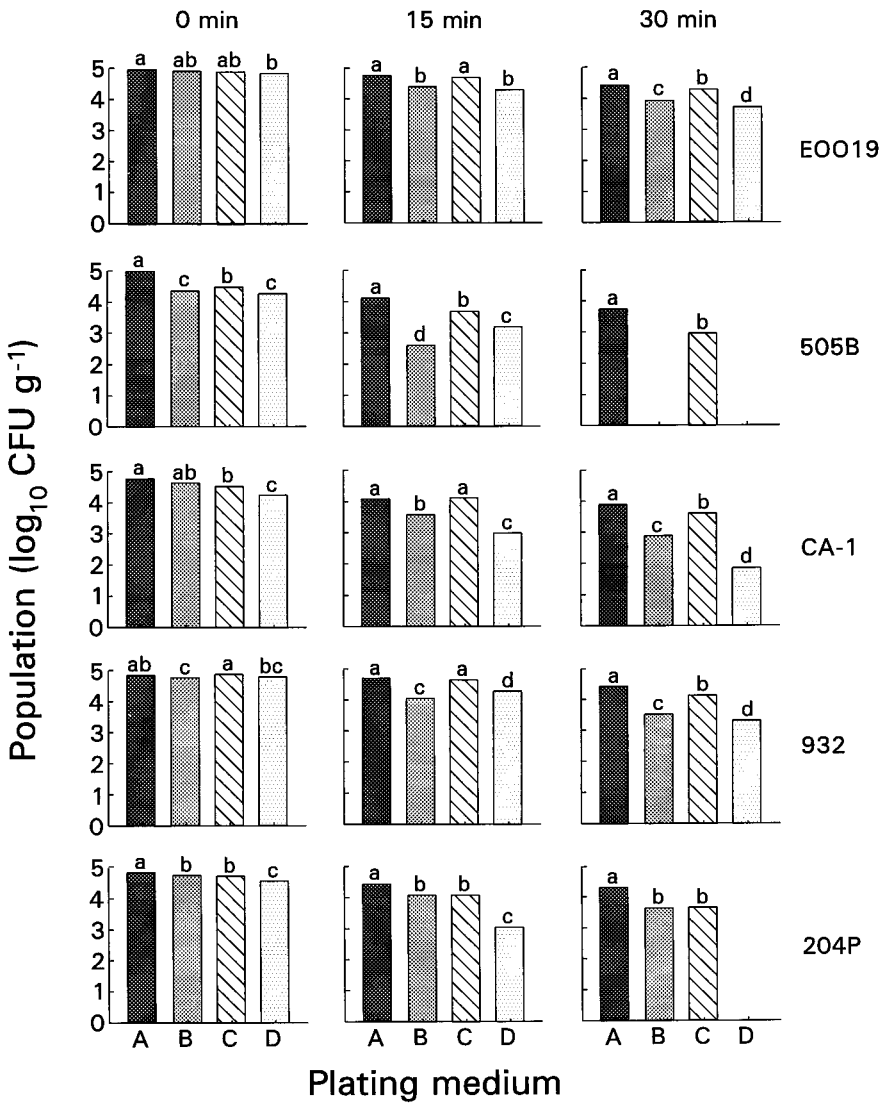

FIG. 2. Recovery of five strains of *E. coli* O157:H7 cells from low-fat ground beef heated at 56°C for 0, 15, and 30 min and plated on TSA (A), MSMA (B), MEMB agar (C), and MSD agar (D). Values for the same strain and heat treatment not showing the same lowercase letter are significantly different ($P \leq 0.05$).

heated low- and high-fat ground beef was assessed. Because of differences in heat transfer characteristics of TSB and ground beef, a higher treatment temperature (56°C) was used for the ground beef study. Results of tests with low-fat beef are illustrated in Fig. 2. Regardless of the strain and heating time, TSA supported the highest recovery of cells. TSA is nonselective and therefore supports the growth of other microorganisms in beef. Initial microbial populations in beef were in the range of 2.39 to 2.56 $\log_{10}$ CFU g^{-1}. Beef was ground as aseptically as possible (i.e., use of gloves, a sanitized grinder, and sterile utensils) to keep the microbial populations at lower levels than would normally be present in retail ground beef. Overall, MSD agar performed the most poorly for recovering unheated and heated cells from low-fat ground beef. The visual appearance of *E. coli* O157:H7 colonies on MSMA and MEMB agar was quite distinctive, and colonies easily selected for further biochemical characterization were confirmed as *E. coli* O157:H7.

Strains 505B and CA-1 appeared to be more sensitive to heat than the other strains were. Observations with strain CA-1 were in contrast to those observed when TSB was used as a heating medium (Fig. 1). Differences in composition and/or pH of TSB and ground beef may have influenced rates of inactivation of various strains differently. Recovery of viable cells from heated ground beef was poorer on MSMA than on TSA or MEMB agar. In all cases, recovery of cells from beef heated for 15 min was significantly lower on MSD agar than on MEMB agar. Decreases in populations of *E. coli* O157:H7 recovered on all four enumeration media were observed as the heating time was increased to 30 min. Nonselective TSA supported significantly higher recovery of heat-stressed cells than did the three selective media. Of the three selective media, MEMB agar performed best. Both MSMA and MSD agar failed to recover viable cells of strain 505B from beef heated for 30 min; no viable cells of strain 204P were recovered on MSD agar.

Populations of *E. coli* O157:H7 recovered from low-fat ground beef stored for 0, 1, 14, or 60 days at −20°C and then plated on TSA, MSMA, MEMB agar, and MSD agar are shown in Fig. 3. Except for strain 204P, significant differences in the number of cells recovered from control day 0) ground

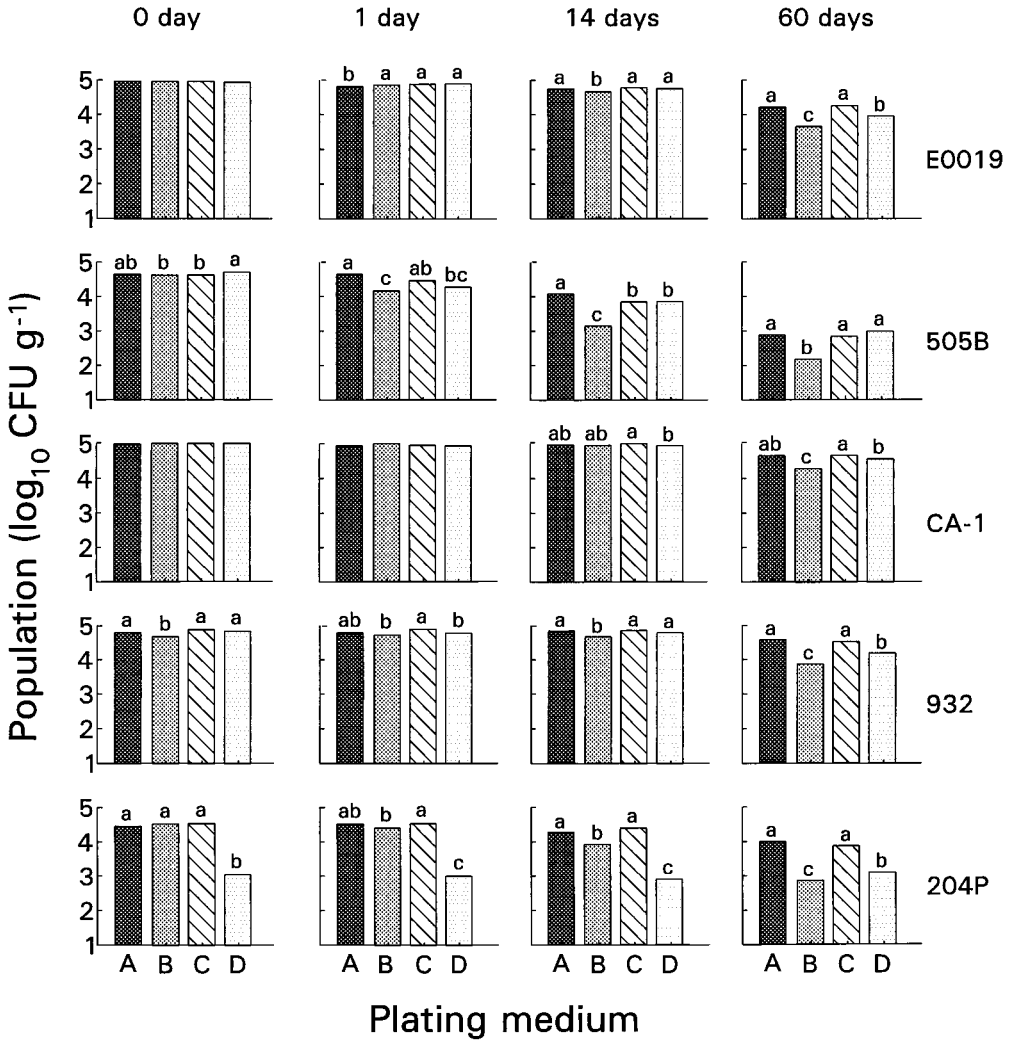

FIG. 3. Recovery of five strains of *E. coli* O157:H7 cells from low-fat ground beef frozen at −20°C for 0, 1, 14, or 60 days and plated on TSA (A), MSMA (B), MEMB agar (C), and MSD agar (D). Values for the same strain and freezing treatment not showing the same lowercase letter are significantly different ($P \leq 0.05$).

beef on TSA and selective media were few. Low recovery of strain 204P cells from low-fat beef stored for 0, 1, or 14 days was obtained for MSD agar compared with the other media. Storage of low-fat beef at −20°C for 1 or 14 days had little or no effect on the viability of cells of strains E0019, CA-1, and 932 and their ability to grow on any of the recovery media. A reduction in populations of strains 505B and 204P was observed with an increase in storage time, suggesting that these strains were more sensitive to freezing. With the exception of strain CA-1 at 14 days, recovery of all strains was significantly lower on MSMA than on TSA or MEMB agar after 14 or 60 days of storage. Regardless of the strain, a reduction in *E. coli* O157:H7 populations occurred in low-fat beef stored for 60 days at −20°C. This decrease in the number of viable cells detected was undoubtedly caused by thawing and repeated freezing at the 14-day sampling time and by the inability of selective media to support growth of stressed cells. The highest recovery was obtained on TSA and MEMB agar, while MSMA gave the poorest recovery.

Populations of *E. coli* O157:H7 recovered from high-fat beef heated at 56°C or frozen at −20°C on TSA and selective media followed the same pattern as that observed for low-fat beef subjected to the same respective treatments. Generally, higher populations were recovered from heated high-fat beef than from low-fat beef, but the relative performance of plating media was the same, regardless of the fat content.

The inability of MSMA to recover heat-stressed *E. coli* O157:H7 is attributed, in part, to the presence of bile salts. Roth et al. (14) reported that the presence of bile salts no. 3 in nutrient agar reduced the number of viable heat-treated *E. coli* SA 603 cells recovered by 50%. Freeze-stressed *E. coli* also exhibits increased sensitivity to bile salts and other selective agents (5). Tollison and Johnson (18) observed that supplementation of tryptic phytone glucose agar with 0.85% bile salt interfered with the enumeration of heat-stressed cells of *Shigella flexneri*. In the present study, the inability of the portion of heat- or freeze-stressed cells of *E. coli* O157:H7 and non-O157 to grow on MSMA is attributed to the lethal effect of bile salts. Regardless of whether the medium in which cells were treated was TSB or ground beef, data clearly demonstrate that injured cells are not able to resuscitate on MSMA. Recovery on nonselective TSA of cells heated in TSB exceeded recovery on MSMA by 1 to 3 log units. On the basis of these observations, detection of small populations of temperature-stressed *E. coli*

O157:H7 in foods would be difficult and would probably yield false-negative results.

Investigations in our laboratory have revealed that the addition of catalase (0.5%), pyruvate (0.5%), or thiodipropionic acid (0.1%) to MSMA did not improve recovery of heat-stressed *E. coli* O157:H7 cells (unpublished data). These chemicals either altered the visual appearance of MSMA or promoted growth of background microorganisms in ground beef. MEMB and MSD agars were therefore formulated and evaluated against MSMA and TSA. It has been reported that heat-stressed *E. coli* loses viability in Levine's eosin methylene blue (EMB) agar (7). Our study has shown that substitution of lactose with sorbitol in EMB agar enhances the recovery of heat- and freeze-stressed *E. coli* O157:H7 cells, as well as heat-stressed *E. coli* non-O157 cells, even when 0.5% NaCl is present as a minor selective agent. It is not known if lactose inhibits repair of stressed *E. coli* O157:H7 cells, but Roth et al. (14) reported that lactose broth does inhibit the growth of some strains of *E. coli*. It is well established that heat-stressed microbial cells exhibit increased sensitivity to NaCl (3, 4, 8).

It was not surprising that the use of sorbitol in place of lactose in EMB agar (MEMB agar) altered the visual appearance of *E. coli* colonies. Instead of the typical greenish, metallic sheen commonly observed on Levine's EMB agar, colonies of *E. coli* O157:H7 were light purple to purple whereas *E. coli* non-O157 colonies were pink. Discrimination between colonies of various serotypes of *E. coli* may require increased experience on the part of the technician. The addition of MUG to MEMB agar caused the area surrounding colonies to fluoresce yellow-green after 24 h of incubation at 37°C. However, after 48 h, which is recommended for the enumeration of colonies that may be formed by stressed cells, the fluorescent compound diffused throughout the medium. This phenomenon suggests that the addition of chromogenic substances may aid in the visual detection of *E. coli* O157:H7 colonies. The diffusivity of MUG has also been noted by Okrend et al. (11). They recommended the use of 4-bromo-5-chloro-3-indolylglucuronide (BCIG) instead of MUG in SMA.

MSD agar also supported better recovery of stressed *E. coli* O157:H7 and non-O157 cells from TSB and frozen ground beef than did MSMA. *E. coli* O157:H7 colonies that formed on MSD agar were pink after 48 h at 37°C, whereas colonies of non-O157:H7 strains were yellow. Although visual differentiation of *E. coli* O157:H7 colonies on MSD agar is easier than on MEMB agar, MSD agar was inferior for recovering the pathogen from heated ground beef. The reason for the difference in performance is not known, but it is possible that cells underwent different degrees of injury when heated in TSB or ground beef or that a component(s) in heated beef reacted with a component in MSD agar to yield a compound that did not permit resuscitation of cells. It has been stated that recovery of freeze-injured *E. coli* is better on complex media containing adequate peptide sources than on minimal media (3). MSD agar meets these requirements.

The results of this investigation further call into question the suitability of SMA for detecting or enumerating *E. coli* O157:H7, especially when cells may be stressed or injured. The increasing incidence of outbreaks of food-borne illness caused by *E. coli* O157:H7 underscores the need to develop better detection and enumeration media. MEMB and MSD agars are better alternatives to MSMA, but their efficacy for recovery of *E. coli* O157:H7 from foods, particularly beef and beef products processed, packaged, and stored under a range of conditions, should be investigated further.

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