

Suitability of Modified Eosin Methylene Blue Agar for Recovering Heat-Injured *E. coli* O157:H7 and *Salmonella* serovars from Cooked Meat Products

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Introduction

- Thermal process lethality is essential in producing safe cooked beef products.
- Conditions during thermal processing may not kill all pathogenic bacteria.
- Injured pathogens have the potential to recover and grow within the cooked meat product after cooling.
- Selective-differential media are often used to enumerate pathogens, but contain harsh ingredients which may prevent colony formation by heat injured cells. In this way, the thermal process lethality may be over-estimated.
- To combat this problem, overlay plating methods have been employed. These methods allow for the recovery of damaged cells on a non-selective medium, followed by overlaying with a selective-differential medium to limit the visible growth to the selected pathogens.
- While overlay plating is a more inclusive enumeration method, it requires more time and materials than direct plating.
- Previous work has shown the potential for increased recovery of heat-injured *Escherichia coli* O157:H7 from ground beef using direct plating on modified eosin methylene blue agar (MEMB; Rochelle et al. 1995. *Appl. Env. Microbiol.* 61: 3268)
- The present study made the following comparisons of methods for enumerating *E. coli* O157:H7 and *Salmonella* in heat-processed meats.

Meat Product	Pathogen	Methods Compared
Cooked ground beef	<i>E. coli</i> O157:H7	MEMB direct, MEMB overlay SMAC direct, SMAC overlay
Cooked jerky	<i>E. coli</i> O157:H7, <i>Salmonella</i>	MEMB direct, XLD overlay

Ground Beef Inoculation

Inoculum Preparation:

- Five strains of outbreak-associated *E. coli* O157:H7 were anaerobically grown for 24 hrs at 35°C, re-suspended in Butterfield's Phosphate Diluent (BPD; Nelson-Jameson, Marshfield, WI), combined and used to inoculate ground beef before cooking.
- Five strains of *Salmonella* serovars and five strains of heat-resistant *E. coli* O157:H7 were grown aerobically for 24 hrs at 35°C, re-suspended in BPD and used to inoculate batter for ground-and-formed beef jerky.

Inoculation :

Ground beef

- Small 25 g bags of lean ground beef were inoculated with 1.0 mL of five-strain *E. coli* O157:H7 inoculum. The inoculum was manually dispersed throughout the meat.

Ground-and-formed beef jerky batter

- 453 g of lean ground beef was placed in 3.785 L Ziploc bags and seasoned, by hand mixing with "Colorado" seasoning (Excalibur Seasoning Company, Pekin, IL) according to manufacturer's directions.
- 8 mL of the ten-strain pathogen cocktail was added to the seasoned ground beef in the Ziploc bag and mixed by hand.
- Jerky batter was placed on sterilized rimmed trays and rolled to a depth of 0.64 cm using a sterilized Nalgene bottle.
- Strips were cut into 2.5 x 12.5 cm strips with a sterilized pizza cutter—yielding 18, 2.5 x 12.5 x 0.64 cm strips per tray.

Cooking Conditions

Ground beef

- Small bags were subjected to three trials each of four simulated slow-cook (405 min) processes in a water bath, set to varying temperatures.
- Ground beef bags were sampled sequentially 9 times per trial over 12 trials (n=108), with beef temperature monitored each minute using a data logger.

Ground-and-formed beef jerky

- After strips were cut they were treated in one of two ways:
- Strips were removed from the tray and placed on racks for processing in a small commercial dehydrator (Pragotrade model TS160, Cabela's Inc., Sidney, NE), or
- Trays were covered in foil and placed in coolers with ice packs for transport to the Alkar-RapidPak Research and Technology Center for Processing in a commercial smokehouse (Model 2000, Alkar, Lodi, WI).
- In total, 6 separate processes were evaluated including: medium temperature (21.1-68.3°C) for extended periods of time (up to 7h); high dry-bulb temperature (76.7°C) with high wet-bulb temperature (71.7°C) for a short time (2h); and long processes (~5h) with increasing dry-bulb temperatures (54.5 to 76.7°C).

Media Preparation

Media Preparation:

- Nutrient agar (NA) was used as the non-selective recovery medium for the cooked ground beef; brain-heart infusion agar (BHIA) was used as the non-selective recovery medium for the ground-and-formed beef jerky.
- Sorbitol MacConkey agar (SMAC) and MEMB were used as the overlay media with cooked ground beef, and xylose lysine desoxycholate agar (XLD) was used as the overlay medium with jerky.
- MEMB was made from lactose-free eosin methylene blue agar which was supplemented with 5 g NaCl and 10 g sorbitol per 1.0 L of agar.
- Sorbitol was added to differentiate sorbitol-fermenting organisms (non-O157:H7 *E. coli* and *Salmonella*) from non-sorbitol-fermenting organisms (*E. coli* O157:H7). NaCl was added for additional selectivity of coliforms.
- NA, BHIA, MEMB, and SMAC used for direct plating were made per manufacturer's directions (Becton Dickinson, Sparks, MD) and poured into sterile 100 x 15 mm plates (Fisher Scientific, Hanover Park, IL).
- XLD, MEMB, and SMAC used for overlays were made per manufacturer's directions and held in the liquid state in a 47°C water bath.

Enumeration of surviving cells:

Cooked ground beef

- Sample bags (n = 108) were held on ice for 30 sec. to stop cooking; the exterior was sterilized with 70% EtOH, aseptically cut to remove the wire closure, and everted into a Whirl-pak filter bag (Nasco, Fort Atkinson, WI).
- Ground beef was then combined with 99 mL BPD and stomached for 30 sec. at medium speed.
- Stomached liquid was diluted accordingly (BPD), spread-plated onto NA, and incubated for injury-repair (60 min. at 35°C). MEMB and SMAC direct-plating omitted the injury-repair step.
- After injury-repair, plates were then overlaid with MEMB or SMAC, held for 1 hr at room temperature and incubated for another 24 hrs at 35°C before counting colonies.

Ground-and-formed beef jerky

- Heated and dried strips (n=176) were stomached individually for 2 minutes at medium speed in 99 mL of BPD, diluted and subsequently direct-plated on BHIA and MEMB.
- BHIA plates were incubated for injury-repair (60 min. at 35°C), overlaid with XLD agar and then allowed to gel for 1 h on the laboratory bench top. MEMB plates were also held for 1 h at room temperature.
- All plates were then incubated at 35°C for 24h before counting colonies.



Figure 1

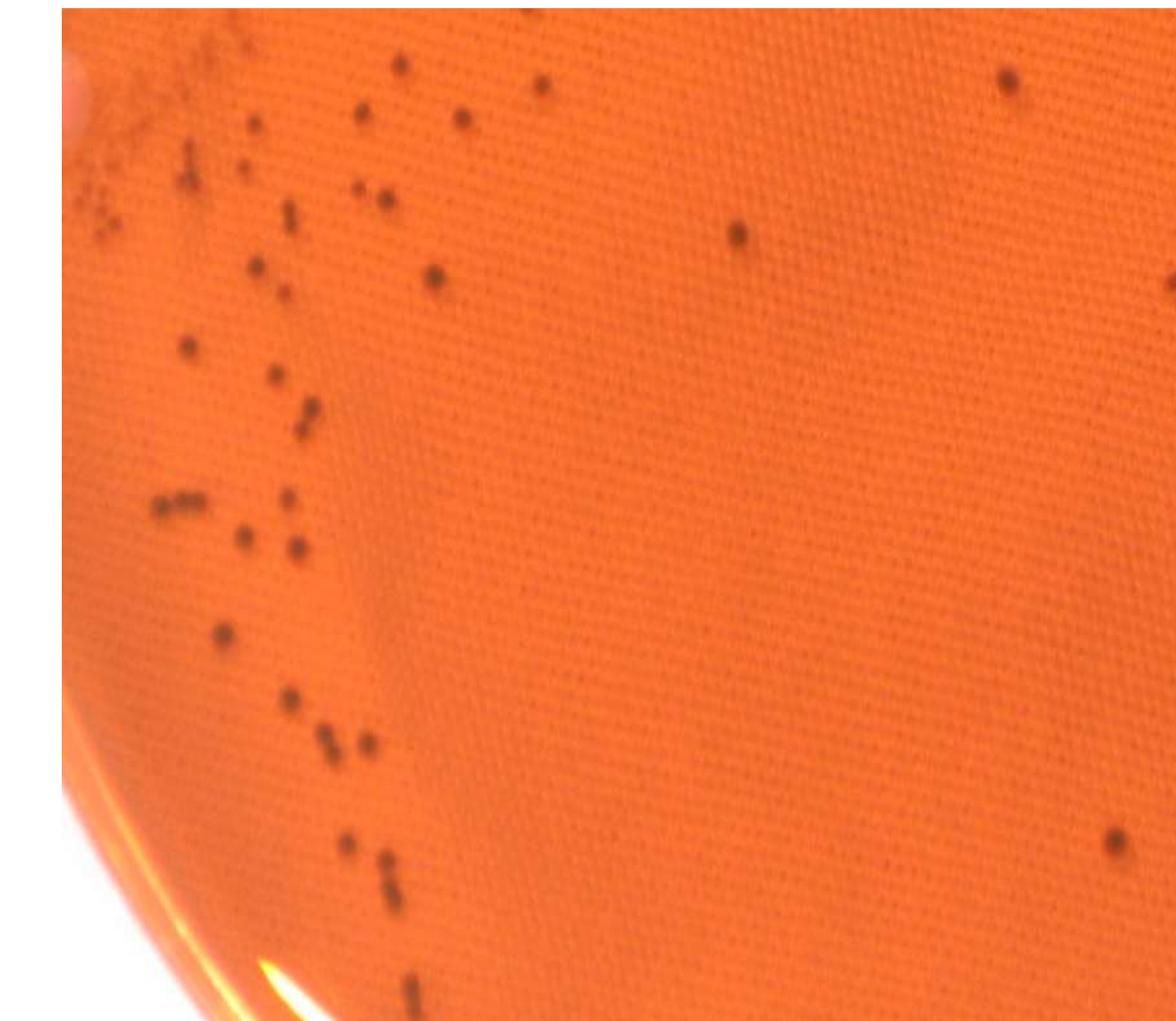


Figure 2

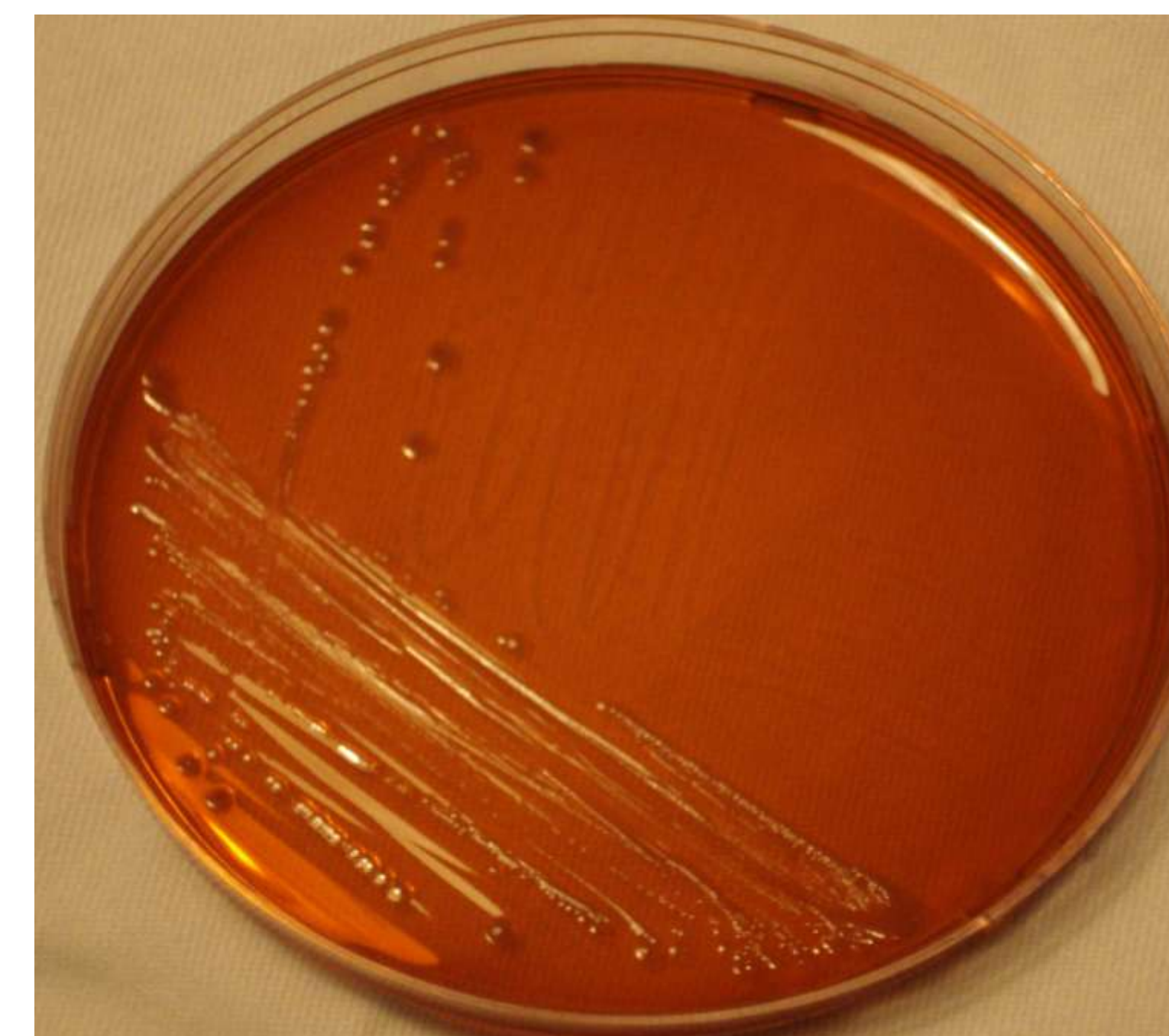


Figure 3

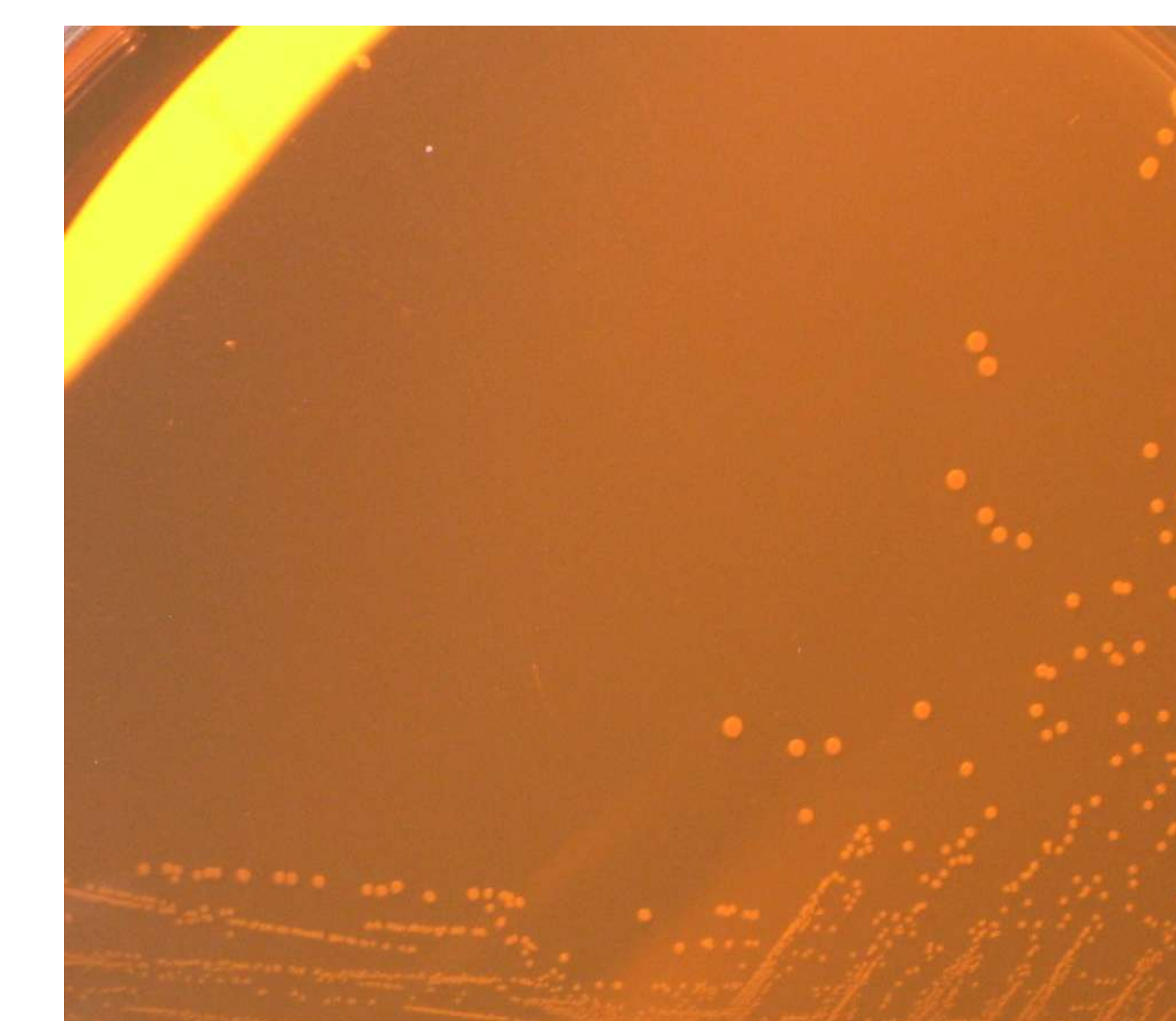


Figure 4



Figure 5



Figure 6

Figure 1: *Salmonella* Typhi on direct plated MEMB. Colonies appear dark pink or purple with iridescent green sheen.

Figure 2: *Salmonella* Typhi on overlay plated MEMB. Colonies appear small and dark pink or purple.

Figure 3: *E. coli* O157:H7 on direct plated MEMB. Colonies appear clear with light pink or purple tint. No iridescence.

Figure 4: *E. coli* O157:H7 on overlay plated MEMB. Colonies appear small and transparent pink.

Figure 5: Generic *E. coli* on direct plated MEMB. Colonies appear shiny, dark pink or purple with or without clear halo. No iridescence.

Figure 6: Generic *E. coli* on overlay plated MEMB. Colonies appear small and dark pink or purple with or without halo.

Statistical Analyses

- Data were analyzed with the PROC MIXED least square means method in SAS 9.2 statistical software (SAS Institute Inc., Cary, NC).
- Analysis of Variance (ANOVA) was performed to determine statistical significance between direct and overlay SMAC and MEMB plating of *E. coli* O157:H7 after cooking of ground beef, and between XLD overlay and direct MEMB plating of *E. coli* O157:H7 and *Salmonella* after cooking/drying of ground-and-formed jerky.
- Differences of least squared means were adjusted using the Tukey and Tukey-Kramer methods to account for sample size discrepancies.
- Significance testing level was fixed at 0.05.

Results and Discussion

- Direct plating on MEMB recovered significantly more *E. coli* O157:H7 and *Salmonella* serovars than SMAC direct plating or XLD overlay plating ($P < 0.05$), respectively.
- Using MEMB as an overlay medium recovered significantly more *E. coli* O157:H7 than direct plating on SMAC ($P < 0.05$).
- No significant difference was found between MEMB overlay and SMAC overlay plating ($P < 0.05$), but there was a consistent trend of greater recovery using the MEMB overlay method.
- Colonies of *E. coli* O157:H7, *Salmonella* spp., and generic *E. coli* are easily distinguishable on directly plated MEMB (Figures 1, 3, 5).
- Colonies are not as distinguishable when using MEMB as an overlay medium if *Salmonella* spp., *E. coli* O157:H7, and generic *E. coli* are all present in the sample because the iridescent sheen is not visible and colony color is more variable (Figures 2, 4, 6).

Conclusions

- MEMB appears to be less harsh than either SMAC and XLD for the recovery of *E. coli* O157:H7 and *Salmonella* serovars thus allowing for better recovery of these pathogens from heated meat products.
- MEMB is versatile in that these pathogens, along with generic *E. coli*, are easily distinguishable when directly plated.
- While not significantly different from SMAC overlay plating in terms of *E. coli* O157:H7 recovery, MEMB overlay plating is useful when a single pathogen is being recovered; however, multiple pathogens or mixed cultures are not easily distinguished on MEMB overlay plates.

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