THE GROWTH OF *PSEUDOMONAS FLUORESCENS* AND NON-PATHOGENIC *E. COLI* IN ASEPTICALLY OBTAINED FRESH GROUND BEEF STORED AT 4°C AND 25°C

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BACKGROUND

The inhibition of pathogens can be due to the competition for many different factors (Ragrubeer *et al.*, 1994). The relationships and possible interactions among microorganisms have been investigated in recent years. It was reported that only at high concentrations, that one species of bacteria would inhibit the growth of others (Palumbo *et al.*, 1997). However, those studies did not explain the interactions of low concentration of different bacteria during the storage period at different temperatures using aseptic fresh ground meat as a media. *Pseudomonas* and *E. coli* were chosen due to the former abundance in meat and the later acting as an indicator for a pathogen. Zero, 2 and 4 logs cells/g of each bacteria inoculum were applied in all combinations to aseptically obtained fresh ground beef which was stored at 4 and 25°C for 14 days or 30 hours.

OBJECTIVES

The objectives of this research was to assist with the understanding of the interactions between microorganisms and to see if the background bacteria can protect meat against pathogens and the possible antagonistic effect of background organisms.

A simplified research was conducted to understand the interactions between different bacteria (*Pseudomonas* and *E. coli*) at different concentrations (zero, 2 and 4 logs) stored at different temperatures (4° and 25°C) using meat as a medium. By utilizing two different temperatures; 4°C (refrigerated temperature) for 14 days and 25°C (room temperature) for 30 hours; a better spectrum of the growth of both *Pseudomonas* and *E. coli* were could be obtained to evaluate the growth rate and interactions involved.

METHODS

Bacterial Cultures and Meat Preparation *Pseudomonas fluorescens* (OSU697) and *E. coli* (OSU XK51) were obtained from the Department of Food Science and Technology of The Ohio State University. Both bacteria were grown for 18 hours in Tryptic Soy Broth (TSB, Difco Laboratories, Detroit, MI) at 35°C. After centrifuging, the final precipitations were diluted with 0.1% (w/v) peptone and measured for optical densities utilizing a Spectronic 20 (Bausch and Lomb) at 600 nm before inoculation into aseptically fresh ground beef.

The outer surface of beef rounds was heated with a hot plate at 400°F for 30 seconds and the inner parts were aseptically removed and ground. Eleven grams of fresh ground meat was then weighed and bagged before inoculation. The inoculation treatments are shown in Table 1. Zero inoculation levels (for control) were diluted with equal amounts of sterile 0.1% peptone water. After inoculation, each bag was stored either at 4 or 25°C for designated sampling times.

Meat storage and Analysis The inoculated samples were evaluated at Day 0, 4th, 7th, 10th and 14th for the 4°C storage; and every 5 hours for the 25°C stored samples at hr 0, 5, 10, 15, 20, 25, 30. Ninety-nine ml vol. of 0.1% peptone water was added to each bag and homogenized in a stomacher for 2 minutes. Appropriate serial decimal dilutions of each homogenized bag were plated. *Pseudomonas* counts was determined with *Pseudomonas* isolation agar (PIA, Difco Laboratories, Detroit, MI) at 25°C for 48 hours; and *E. coli* with Violet red bile agar (VRBA, Difco Laboratories, Detroit, MI) at 35°C for 24 hours.

Statistical analysis All bacterial counts were transformed to logarithms for statistical analyses. Mixed models were utilized for Analysis of Variance using the Statistical Analysis Systems (SAS Institute, 2001).

RESULTS AND DISCUSSIONS

Pseudomonas counts did not change significantly for each treatment from Day 0 to Day 7 at 4°C. *Pseudomonas* counts showed increases only after 10th day at 4°C for both 2 and 4 logs *Pseudomonas* treated groups with zero, 2 and 4 logs of *E. coli* (E0P2, E2P2 and E4P2; E0P4, E2P4 and E4P4). The reason was probably due to low temperature storage at 4°C that delayed the growth of *Pseudomonas*. No significant differences in Violet Red Bile Agar (VRBA) counts were found for 2 or 4 log *E. coli* inoculated treatments with either zero, 2 or 4 logs of *Pseudomonas* (E2P0, E2P2 and E2P4; E4P0, E4P2 and E4P4) within 7 days of storage at 4°C; however, at Day 10, the VRBA counts decreased but increased at Day 14 for all 9 treatments.

Pseudomonas and *E. coli* numbers increased as storage hour increased ($p<0.05$) after 10 hour at 25°C storage. All the treatments with either zero, 2 or 4 logs of *E. coli* inoculation (E0, E2 and E4) were still significantly different from each other for the VRBA counts after 5 hours storage at 25°C. As time increased, the difference of E2 and E4 group decreased when stored at 25°C. The treatments with 4 log *Pseudomonas* even with different *E. coli* inoculation levels (E0P4, E2P4 and E4P4) did not differ in their PIA counts for the whole 30 hours of storage at 25°C. The same results were observed for treatments stored at 4°C. This indicated the treatments with 4 log *Pseudomonas* inoculation dominated the total microbial population disregarding the presence for even the same level of *E. coli* at both 4 and 25°C.

The growth of 2 and 4 logs *E. coli* from both storage temperatures (4 and 25°C) at each storage period were shown on Figures 1 to 4 by the different *Pseudomonas* inoculation levels (zero, 2 and 4 logs *Pseudomonas*). Both VRBA counts slightly decreased for 2 log *E. coli* with zero, 2 and 4 logs *Pseudomonas* (E2P0, E2P2 and E2P4) (Figure 1) and for 4 log *E. coli* with zero, 2 and 4 logs *Pseudomonas* (E4P0, E4P2 and E4P4) (Figure 2) groups after 10 days storage at 4°C but increased at Day 14. VRBA counts from 2 log *E. coli* with zero, 2 and 4 log *Pseudomonas* (E2P0, E2P2 and E2P4) (Figure 3) and 4 log *E. coli* with zero, 2 and 4 log *Pseudomonas* (E4P0, E4P2 and E4P4) (Figure 4) increased as time increased until Hour 25 but decreased at Hour 30 (except for E2P0; 2 log *E. coli* with zero *Pseudomonas*) at 25°C storage.

CONCLUSIONS

Under both storage temperatures (4 and 25°C), *Pseudomonas* grew faster than *E. coli*. *E. coli* showed little growth (even slight decreased in VRBA numbers) under 4°C storage until Day 14. No decreases of *E. coli* were observed at the end of day 14 under 4°C; but the VRBA numbers decreased after 30 hour at 25°C storage. The reason is probably due to the higher numbers of bacteria growing at 25°C and at hour 30, where the whole population was approaching the death phase of growth. However, the PIA counts at Hour 30 did not decreased as much as VRBA counts for all treatments. Therefore, the interaction would suggest that inhibition occurred for *E. coli* by high numbers of *Pseudomonas* at Hour 30 at 25°C storage. At that time, the meat was spoiled with higher than 10^7 CFU/g in TPC numbers. Therefore, the interactions between the two species were not of consumer importance even with a cross design of different levels of bacteria strains. Storage temperatures and/or the length of lag phase of microorganisms seem to be a much bigger factor in affecting the bacteria growth in this study.

PERTINENT LITERATURE

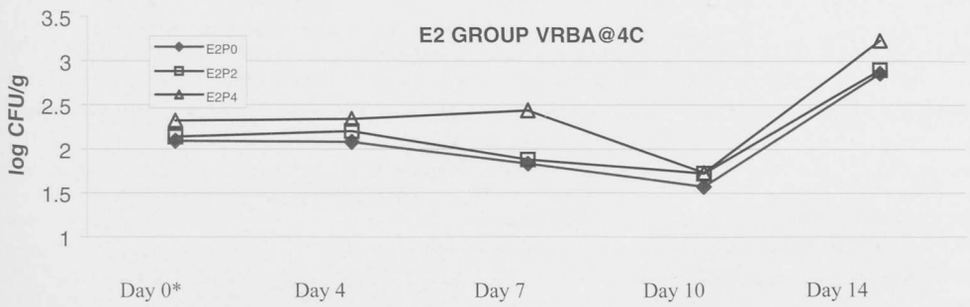
Palumbo, Samuel A.; Allan Pickard and Jeffrey E. Call 1997. "Population Changes and Verotoxin Production of Enterohemorrhagic *Escherichia coli* Strains Inoculated in Milk and Ground Beef Held at Low Temperatures." Journal of Food Protection 60(7) 746-750.

Raghubeer, Errol V.; Jim S. KE; Michael L. Campbell and Richard S. Meyer 1994. "Fate of *Escherrichia coli* O157:H7 and Other Coliform in Commercial Mayonnaise and Refrigerated Salad Dressing." Journal of Food Protection 58(1)13-18.

Table 1 Treatment inoculation plan for both 4°C and 25°C.

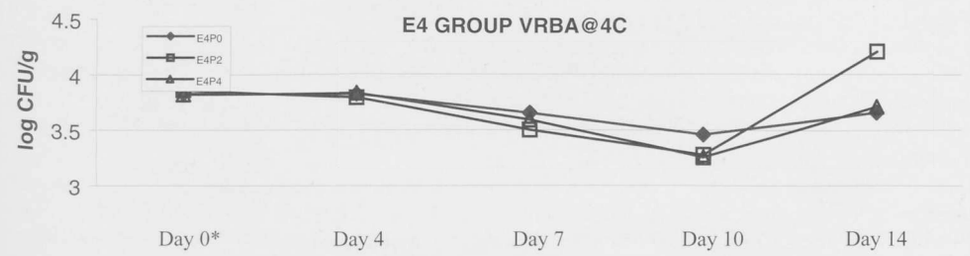
Treatment	Symbol	Inoculation levels (log cells/gm of meat)	
		E. coli	*Pseudomonas*
1	E0P0	0	0
2	E0P2	0	2
3	E0P4	0	4
4	E2P0	2	0
5	E2P2	2	2
6	E2P4	2	4
7	E4P0	4	0
8	E4P2	4	2
9	E4P4	4	4

Figure 1. Violet Red Bile Agar (VRBA) numbers of 2 log level of *E. coli* inoculation at 4°C.



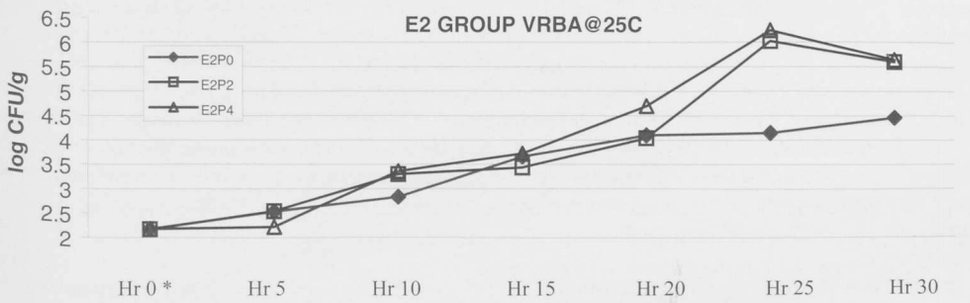
*Storage days
*E2P0 means the treatment had 2 log *E. coli* and zero *Pseudomonas* inoculation.
*E2P2 means the treatment had 2 log *E. coli* and 2 log *Pseudomonas* inoculation.
*E2P4 means the treatment had 2 log *E. coli* and 4 log *Pseudomonas* inoculation.

Figure 2. Violet Red Bile Agar (VRBA) numbers of 4 log level of *E. coli* inoculation at 4°C.



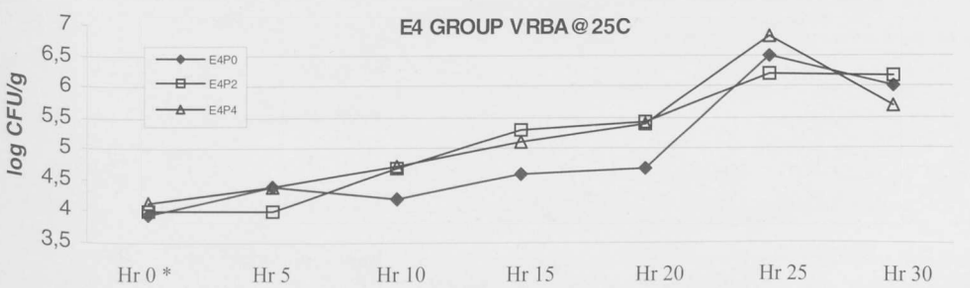
*Storage days
*E4P0 means the treatment had 4 log *E. coli* and zero *Pseudomonas* inoculation.
*E4P2 means the treatment had 4 log *E. coli* and 2 log *Pseudomonas* inoculation.
*E4P4 means the treatment had 4 log *E. coli* and 4 log *Pseudomonas* inoculation.

Figure 3. Violet Red Bile Agar (VRBA) numbers of 2 log level of *E. coli* inoculation at 25°C.



*Storage Hours (Hr)
*E2P0 means the treatment had 2 log *E. coli* and zero *Pseudomonas* inoculation.
*E2P2 means the treatment had 2 log *E. coli* and 2 log *Pseudomonas* inoculation.
*E2P4 means the treatment had 2 log *E. coli* and 4 log *Pseudomonas* inoculation.

Figure 4. Violet Red Bile Agar (VRBA) numbers of 4 log level of *E. coli* inoculation at 25°C.



*Storage Hours (Hr)
*E4P0 means the treatment had 4 log *E. coli* and zero *Pseudomonas* inoculation.
*E4P2 means the treatment had 4 log *E. coli* and 2 log *Pseudomonas* inoculation.
*E4P4 means the treatment had 4 log *E. coli* and 4 log *Pseudomonas* inoculation.