THE INFLUENCE OF *PSEUDOMONAS FLUORESCENS* AND *LACTOBACILLUS PLANTARUM* GROWTH ON *ESCHERICHIA COLI* IN ASEPTICALLY-PREPARED FRESH GROUND BEEF STORED AT 4 AND 25 °C

Yi-Mei Sun and Herbert W. Ockerman

Department of Animal Sciences, The Ohio State University, 2029 Fyffe Road, OH 43210, USA

Background

It is suggested that applications of predictive models on food should be re-evaluated since the bacteria's behavior is different on different media (bacterial diffusion coefficient is smaller in solid food; Dens and Van Impe, 2001). This experiment investigated the effect of LAB (lactic acid bacteria) strain (*Lactobacillus*) along with the major background bacteria (*Pseudomonas*) in meat on the microbiological growth of *E. coli* utilizing aseptically-separated fresh ground beef (solid food) at two different storage temperatures (4 and 25 °C).

More reductions of *E. coli* with higher *Pseudomonas* inoculation were observed in this experiment but not in previous experiments (with only *E. coli* and *Pseudomonas*) when meat was stored at 4 °C. The results implied that the addition of *Lactobacillus* had a synergistic effect on reducing *E. coli* at 4 °C storage. It also suggested the probability that more strains of background bacteria along with refrigerated storage would result in more interactions between species and probably better inhibition effects on the growth of fecal source bacteria.

Objectives

The objectives of this research was to assist with the understanding of the interactions between microorganisms and to help evaluate if more varieties of background bacteria can protect meat against colonization from fecal contamination (or pathogens) and the possible antagonistic effect from background organisms in aseptically-prepared fresh ground beef stored at 4 and 25 °C.

Methods

Bacterial cultures and meat preparation and inoculation

Pseudomonas fluorescens (OSU 697) and *E. coli* (OSU XK51) were grown for 18 hours in Tryptic Soy Broth (TSB, Difco Laboratories, Detroit, MI) at 35°C while *Lactobacillus plantarum* (OSU 33) was grown in Lactobacilli MRS (deMan Rogosa &Sharpe) broth at 35°C. After incubation, cultures were centrifuged at 903 x *g* and proper concentrations were measured utilizing optical densities at 600 nm (final concentration of 2 log and 4 log cells/gm of meat were determined) before inoculation into aseptic fresh ground beef. Each outer surface of beef rounds (at approximately 14 days of post mortem) was heated with a hot plate at 450°F (232°C) for 30 seconds. The external parts were aseptically removed and the internal parts ground.

The inoculation plan for treatments is shown in Table 1. Zero inoculation levels were diluted with equal amounts of sterile 0.1% peptone water. After inoculation, tissues were then stored either at 4 or 25°C for the designated sampling times. The inoculated samples were evaluated at 0, 4th, 7th, 10th and 14th days at 4°C and evaluated at 0, 5th, 10th, 15th, 20th, 25th, and 30th hours when stored at 25°C. *Pseudomonas* counts was determined with *Pseudomonas* isolation agar (PIA, Difco) at 25°C for 48 hours and *E. coli* was determined with Violet red bile agar (VRBA, Difco) at 35°C for 24 hours. MRS agar was used to determine the growth of *Lactobacillus* (Lactobacilli MRS broth with 1.5% agar, Difco) at 35°C for 48 hours.

Statistical analysis

All bacterial counts were transformed to logarithms for statistical analyses. The experimental design was a randomized complete block design (experimental design) with two treatment designs of 3 x 3 x 3 x time factorial designs conducted for different temperatures. General Linear Model (GLM) was used for Analysis of Variance (SAS Institute, 2001). Least square means were chosen for mean separations (Harvey, 1982).

Table 1. Treatment inoculation plans and descriptions (*Pseudomonas*, *E. coli* and *Lactobacillus* stored at both 4 °C and 25 °C).

		Inoculation levels (log cells/gm of meat)		
Treatment	Symbol*	E. coli	Lactobacillus	Pseudomonas
1	E0L0P0	0	0	0
2	E2L0P0	2	0	0
3	E4L0P0	4	0	0
4	E0L2P0	0	2	0
5	E2L2P0	2	2	0
6	E4L2P0	4	2	0
7	E0L4P0	0	4	0
8	E2L4P0	2	4	0
9	E4L4P0	4	4	0
10	E0L0P2	0	0	2
11	E2L0P2	2	0	2
12	E4L0P2	4	0	2
13	E0L2P2	0	2	2
14	E2L2P2	2	2	2
15	E4L2P2	4	2	2
16	E0L4P2	0	4	2
17	E2L4P2	2	4	2
18	E4L4P2	4	4	2
19	E0L0P4	0	0	4
20	E2L0P4	2	0	4
21	E4L0P4	4	0	4
22	E0L2P4	0	2	4
23	E2L2P4	2	2	4
24	E4L2P4	4	2	4
25	E0L4P4	0	4	4
26	E2L4P4	2	4	4
27	E4L4P4	4	4	4

* E: *E. coli*, L: *Lactobacillus* and P: *Pseudomonas*
* E2L4P2: 2 log *E. coli*, 4 log *Lactobacillus* and 2 log *Pseudomonas* inoculated, etc.

Results and Discussion

Violet red bile agar (VRBA) utilized for *E. coli* counts at 4°C

Treatments with *Pseudomonas* inoculation (P2 and P4) caused significant reductions ($p < 0.05$) in VRBA (*E. coli*) counts compared to treatments without *Pseudomonas* inoculation (P0) when stored at 4°C (Table 2). It indicated that higher *Pseudomonas* levels inhibited the growth of *E. coli* (reduction in VRBA counts) more than lower levels or zero *Pseudomonas* which agrees with Jay's hypothesis in 1996 (higher numbers of background bacteria will inhibit the growth of *E. coli* more than lower numbers of background bacteria).

Decreasing trends ($p<0.05$) in VRBA (*E. coli*) counts were found for both treatments with 2 and 4 logs of *E. coli* inoculation (E2 and E4) during 14

days of storage at 4°C. At Day 7, treatments with 4 log *E. coli* inoculation (E4) began to show a significant decrease in VRBA (*E. coli*) counts compared to those at Day 0 and Day 4 during 14 days of storage at 4°C storage (4.01 to 2.82 logs CFU/g). However, treatments with 4 log *E. coli* inoculation maintained higher VRBA counts throughout the whole 14 days of storage at 4°C even though it had more reductions in VRBA counts during storage when compared to treatments with 2 logs of *E. coli* inoculation. The results are shown on Table 3.
Violet red bile agar (VRBA) utilized for *E. coli* counts at 25°C

Different from the results at 4°C storage, the treatments with 4 log *Pseudomonas* inoculation (P4) had higher VRBA (3.66 logs *E. coli* CFU/g) counts than those of zero or 2 log *Pseudomonas* (P0: 3.02 logs *E. coli* CFU/g and P2: 3.12 logs *E. coli* CFU/g) when stored at 25°C for 30 hours. It appears that 4 log *Pseudomonas* enhanced the growth of *E. coli* when stored at 25°C. Treatments with zero *E. coli* inoculation (E0) maintained a relative low counts due to minor contamination (around 2 logs CFU/g, which is equal to 100 CFU per gram of meat at Hour 25 and 30) during the whole 30 hours when stored at 25°C. Treatments with 2 or 4 logs of *E. coli* inoculation (E2 and E4) showed increases in VRBA (*E. coli*) counts at Hour 15 and kept increasing as storage time increased (approximately 6 logs *E. coli* CFU/g at Hour 25 and 30) when stored at 25°C.

This decrease of *E. coli* growth at 4°C could be due to the higher *Pseudomonas* level which resulted in greater inhibitions on *E. coli* counts in addition to low temperature. However, the higher the *Pseudomonas* inoculation levels, the higher the *E. coli* counts when stored at 25°C. The reason could be due to metabiosis (ready-to-use nutrients that had been degraded by *Pseudomonas*) and the depletion of oxygen by *Pseudomonas* (Gram et al.,2002) that allow *E. coli* to grow better at its preferable temperature.

Conclusions

The inhibition of *E. coli* by *Pseudomonas* along was not obvious in previous experiments but was observed when an additional strain (LAB) was present. The existing of other strains of bacteria will affect the growth of each other, but the effects of storage temperature were also very significant. Therefore, the inhibition of *E. coli* strain with low storage temperature along with multiple bacteria strains co-existing could be a way to inhibit the numbers of unwanted bacteria.

In our research, total exclusion of *E. coli* was not achieved either at 4°C for 14 days or 25°C for 30 hours. Cold sterilization by irradiation (gamma irradiation; Roller, 1999) might be the only method for totally eliminating the existing *E. coli* or other pathogens (free of microbial contamination) in fresh, uncooked meat. Balancing between spoilage (high background microorganisms, mixtures) and safety (lower background microorganisms from trimming, cleaning of meat that allow pathogens like *E. coli* having a better chance to multiple) is difficult. Thus, the results would suggested that higher background bacteria can be used as a biological preservation hurdle in inhibiting *E. coli* growth when stored at 4°C refrigerated temperature.

Table 2. Violet Red Bile Agar (VRBA; *E. coli*) counts* influenced by different *Pseudomonas* inoculation levels averaged across different *Lactobacillus*, *E. coli* inoculation levels, and storage hours when stored at 4 °C

Violet red bile agar (VRBA) counts (*E. coli*) at 4 °C*

	Pseudomonas Inoculation Levels (cells/grams of meat**)		
	0	10^2	10^4
VRBA (CFU/g)	2.34 ± 0.07^{A}	2.14 ± 0.07^{B}	1.87 ± 0.07^{C}

* Data shown as Least Square Mean ± Standard Error (log CFU/g) of VRBA counts. Counts are illustrated as $\log_{10}$ numbers, CFU of *E. coli* counts/g.
^{A,B,C} Different letters of superscript mean significant difference in the same row ($p<0.05$).

Table 3. Violet Red Bile Agar (VRBA; *E. coli*) counts for different *E. coli* inoculation levels and storage time averaged across different *Pseudomonas* and *Lactobacillus* inoculation levels when stored at 4 °C

Violet red bile agar (VRBA) counts (*E. coli*) at 4 °C*

	E. coli Inoculation Levels (cells/grams of meat**)		
Storage Days	0	10^2	10^4
0	0.38 ± 0.16^{Aa} (Est.)	2.45 ± 0.16^{Ba}	4.01 ± 0.15^{Ca}
4	0.90 ± 0.15^{Ab} (Est.)	2.42 ± 0.15^{Bab}	3.82 ± 0.15^{Ca}
7	0.82 ± 0.15^{Ab} (Est.)	2.10 ± 0.15^{Bab}	3.28 ± 0.15^{Cb}
10	0.65 ± 0.15^{Aab} (Est.)	2.01 ± 0.15^{Bb}	3.03 ± 0.15^{Cbc}
14	1.02 ± 0.15^{Ab} (Est.)	1.98 ± 0.16^{Bb}	2.82 ± 0.15^{Cc}

* Data shown as Least Square Mean ± Standard Error (log CFU/g) of VRBA counts. Counts are illustrated as $\log_{10}$ numbers, CFU *E. coli* counts/g.
^{a,b,c} Different letters of superscript mean significant difference in the same column ($p<0.05$).
^{A,B,C} Different letters of superscript mean significant difference in the same row ($p<0.05$).
Est. = Estimated counts VRBA counts at E0 levels indicated contaminations.
** Cells/g of meat = samples final concentrations after inoculations with indicated bacteria.

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