Efficacy of Peracetic Acid from Perasan MP-2 against non-O157:H7 Pathogenic *Escherichia coli*

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Background

On September 13, 2011 the U.S. Department of Agriculture announced that new steps will be taken to further safeguard the food supply in the USA. Six additional Shiga-toxin producing *E. coli* serogroups, or non-O157 STEC, will be declared adulterants if detected in raw ground beef, its components, or tenderized steaks. If any of the *E. coli* serogroups O26, O103, O45, O111, O121 or O145 are detected in raw ground beef, or precursor cuts, those products will be prohibited from sale to consumers. The FSIS will begin enforcing this new policy March 5, 2012.

Currently all establishments producing raw beef products are required to have one or more critical control points in their HACCP plans to eliminate or to reduce *E. coli* O157:H7 below detectable levels. With this new regulation, these establishments will need to reassess their HACCP plans to include the six non-O157 *E. coli* serogroups. Previous studies performed by Enviro Tech Chemical Services demonstrate that peracetic acid (from Perasan MP-2) is efficacious against *E. coli* O157:H7, but the purpose of this study is to observe the sensitivity of these six serogroups compared to *E. coli* O157:H7 by using a low concentration of the antimicrobial. A low level of the peracetic acid (PAA) was chosen in this challenge test to allow the investigators to observe and track the relative efficacy of each serovar over time in the presence of the oxidizer.

Experimental Methods

Serovars Tested:

- *E. coli* O157:H7 (ATCC 35150)
- *E. coli* O26 (ATCC 12795)
- *E. coli* O103 (ATCC 23982)
- *E. coli* O45 (obtained from USDA-ARS-NPA, US Meat Animal Research Center, Clay Center, NB)
- *E. coli* O111 (ATCC 33780)
- *E. coli* O121(obtained from USDA-ARS-NPA, US Meat Animal Research Center, Clay Center, NB)
- *E. coli* O145 (obtained from USDA-ARS-NPA, US Meat Animal Research Center, Clay Center, NB)

Each of the above mentioned test systems were cultured in sterile nutrient broth (Sigma, St. Louis, MO) by incubation for two days at 35^{o} C. The bacteria were separated from the nutrient broth by centrifugation and carefully resuspended in just over one liter of sterile phosphate buffer. The amount of *E. coli* bacteria was measured by serial dilution and plating onto 3M *E. coli* Petrifilms. Each solution was then split into two 500 ml samples. 0.5 ml of **fetal bovine serum** (FBS) was added to one of the two samples (0.1% FBS in solution).

A 1000 mg/L PAA stock solution was prepared by adding 6.45 g of PAA (Perasan MP-2) up to 1000 g of reverse osmosis water. One at a time, the 500 ml samples were treated with a nominal dose of 5 ppm (mg/L) PAA by introducing 2.5 ml of the 1000 mg/L stock solution. Immediately thereafter, the actual concentration of the test material was measured using the DPD colorimetric method. Approximately every two minutes, a calculated amount of the concentrated stock solution was added to each of the test solutions to replenish the PAA that had been depleted. The PAA concentration was tracked throughout a 20 minute time interval.

After 2, 10 and 20 minutes a 20 ml aliquot was removed from the sample. The PAA remaining in the 20 ml aliquot was neutralized by adding
230 µL of a 0.1% erythorbic acid solution. The amount of viable *E. coli* bacteria remaining in the neutralized aliquot was measured by serial dilution and plating on 3M *E. coli* Petrifilms. All

the Petrifilms were incubated at 35° C for 24 hours before enumeration and comparison with the untreated control (plated previous to splitting into two 500 ml samples and challenging with PAA).

This study was performed in duplicate on two separate days, and the average of the results for all plating and challenges is reported below. In addition, all challenge testing was performed at pH values of 6.8-7.2.

Results and Discussion

There was no significant difference between samples that contained FBS and those that did not; as a result, only the data for the samples containing the FBS (organic matter) is reported below.

During the challenge testing, the residual PAA was measured initially and at the 2, 10, 15 and 20 minute time intervals. This data is reported in Table 1. The PAA concentration steadily declined throughout the test period and additional concentrated stock solution was added to replenish the PAA that had been consumed.

Table 1: PPM of Oxidant: Initial (5 mg/L as PAA) nominal dose of PAA followed by tracking the depletion with time and level following replenishment

Time	**E. coli O157:H7 /mg/L as PAA**	**E. coli O26 /mg/L as PAA**	**E. coli O103 /mg/L as PAA**	**E. coli O45 /mg/L as PAA**	**E. coli O111 /mg/L as PAA**	**E. coli O121 /mg/L as PAA**	**E. coli O145 /mg/L as PAA**
Initial	4.28	6.21	5.78	6.42	5.35	5.03	4.49
2 min	4.50	5.46	5.67	3.75	4.60	3.86	4.28
10 min	5.03	5.24	5.24	4.90	4.49	5.56	4.28
15 min	5.14	4.71	5.24	5.03	3.96	5.56	4.60
20 min	4.71	3.85	5.14	5.46	5.14	5.56	5.03

Table 2 shows the remaining $\log_{10}$ CFU/ml of *E. coli* O157:H7 and the six non-O157 *E. coli* samples before treatment with, and maintenance to around 5 mg/L as PAA, at 2, 10, and 20 minutes.

Table 2: Log_{10} CFU/ml *E. coli* serogroups **remaining** in solution over time

Time	***E. coli* O157:H7 /log_{10} CFU/ml**	***E. coli* O26 /log_{10} CFU/ml**	***E. coli* O103 /log_{10} CFU/ml**	***E. coli* O45 /log_{10} CFU/ml**	***E. coli* O111 /log_{10} CFU/ml**	***E. coli* O121 /log_{10} CFU/ml**	***E. coli* O145 /log_{10} CFU/ml**
Control	**6.16**	**8.51**	**6.46**	**7.20**	**5.84**	**6.85**	**6.54**
2 min	6.11	3.17	0.00	3.08	0.00	2.31	4.28
10 min	0.95	0.00	0.00	0.00	0.00	0.00	0.00
20 min	0.00	0.00	0.00	0.00	0.00	0.00	0.00

The log_{10} reduction, the reciprocal of the CFU's remaining, in comparison to the untreated control of each serogroup can be seen in Table 3.

Table 3: Log_{10} **reduction** of the *E. coli* serogroups over time

Time	**E. coli O157:H7 /log_{10} CFU/ml Reduction**	**E. coli O26 /log_{10} CFU/ml Reduction**	**E. coli O103 /log_{10} CFU/ml Reduction**	**E. coli O45 /log_{10} CFU/ml Reduction**	**E. coli O111 /log_{10} CFU/ml Reduction**	**E. coli O121 /log_{10} CFU/ml Reduction**	**E. coli O145 /log_{10} CFU/ml Reduction**
2 min	0.05	5.34	>6.46	4.12	>5.84	4.54	2.26
10 min	5.21	>8.51	>6.46	>7.20	>5.84	>6.85	>6.54
20 min	>6.16	>8.51	>6.46	>7.20	>5.84	>6.85	>6.54

It can be seen that all of the non-O157 serogroups were eradicated at or before the ten minute contact time. Two serogroups (O103 and O111) were eradicated at or before the two minute interval after dosing with PAA. Thus, these two serogroups, O103 and O111, were the next most sensitive to PAA (5 mg/L) followed by O145, O45, O121 and O145, while O157:H7 proved to be the most resistant to this dose after 20 minutes of contact time.

Consequently, in decreasing sensitivity to a dose of 5 mg/L as PAA over a span of 20 minutes, the order is:

O103 ≈ O111 < O26 ≈ O121 ≈ O145 ≈ O45 < O157:H7

Conclusions

- The R&D department of Enviro Tech Chemical Services has performed several efficacy studies using peracetic acid (from Perasan MP-2) against *E. coli* O157:H7 for meat processing facilities and FCN applications. This new regulation going into effect March 2012 has sparked the interest of several of these facilities who are wondering whether antimicrobials are efficacious against these six serogroups, and what is the comparison to *E. coli* O157:H7. Therefore, the purpose of this study was to determine the sensitivity of these six serogroups compared to *E. coli* O157:H7 by using a low concentration of peracetic acid (from Perasan MP-2).
- Two of the serogroups (O103 and O111) were eradicated at or before the two-minute contact time
- The remaining four non-O157 serogroups (O26, O121, O145, and O45) were eradicated at or before the ten-minute contact time with PAA. *E. coli* O157:H7 proved to be the most resistant to this dose, as it was eradicated between 10 and 20 m inutes.
- Thus, in decreasing sensitivity to a dose of 5 mg/L as PAA over a span of 20 minutes, the order is:

O103 ≈ O111 < O26 ≈ O121 ≈ O145 ≈ O45 < O157:H7