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Project Title: Additive effect of Bacteriophage Cocktail and Novel Antimicrobials for Reduction of *Escherichia coli* O157:H7 on Artificially Contaminated Beef Trim

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Objective: This study was designed to determine the efficacy and suitability of EcoShield™ bacteriophage as an antimicrobial intervention for *Escherichia coli* O157:H7 in ground beef as a standalone treatment or as a part of multiple hurdle approach.

Method: A total of 30 beef trims were used for each replication and all experiments were repeated at least three times. Samples (10x10x5 cm, 25-35% fat) were artificially contaminated with *E. coli* O157:H7 by spot inoculating one side of each to achieve an *E. coli* O157:H7 concentration of approximately 10⁵ CFU/g. Pathogens were allowed to attach for one hour at 4°C. After attachment, a set of two beef trims were subjected to one of five antimicrobial treatments; near neutral pH EO water (NEO) with 50 mg/L available chlorine and pH 6.5, acidic pH EO water (EO) with 50 mg/L available chlorine and pH 2.7, 2% levulinic acid plus 0.2% sodium dodecyl sulfate (LSDS) at pH 2.8, 5% lactic acid (LA) at pH 2.04 or EcoShield™ bacteriophage (P-phage treatment was applied using air brush to achieve a phage concentration of approximately 10⁵ PFU/g) alone. Alternatively, spray treatment followed by phage treatments (NEOP, EOP, LAP, and LSDSP) or phage treatment followed by one of the spray treatments (PNEO, PEO, PLA and PLSDS) were also used in this study. At the end of the treatment period, treated trim samples corresponding to each treatment were ground and stored in Styrofoam containers with absorbing pads and sealed with polyvinyl chloride film. All ground beef samples were stored at 4°C and analyzed for presence of *E. coli* O157:H7 and bacteriophage on days 1, 3, 5 and 7.

Results: In this study, the 13 spray treatments with and without bacteriophage on beef trim resulted in reductions of *E. coli* O157:H7 ranging from 54.60 to 95.30 % when compared to non-treated control beef trims from day 1 (Table 1). All wash treatments when combined with

bacteriophage resulted in a higher bacterial reduction compared to corresponding wash treatment alone. Several combination treatments (NEOP, LAP and LSDSP) were able to reduce significantly more *E. coli* O157:H7 from ground beef than EO or NEO treatments alone. Bacteriophage recoveries from samples were between 5.39 to 4.72 log PFU/g.

Application: Results reveal that *E. coli* O157:H7- specific phage, when combined with EO water, LA-SDS or lactic acid could be used as an intervention to reduce levels of the targeted pathogen from ground beef.

Table 1: Reduction of *E. coli* O157:H7 from ground beef via bacteriophage and various wash solutions

		<i>E. coli</i> O157:H7 reduction %* ⁺			
		Day-1	Day-3	Day-5	Day-7
Washing treatments	EO	62.24	69.86	77.24	75.73
	NEO	54.60	63.35	73.02	72.33
	LA	83.66	84.89	87.92	88.95
	LSDS	77.86	82.93	88.14	87.66
	P	65.08	76.66	84.65	84.65
Washing treatments followed by phage	EOP	90.06	85.71	91.48	90.71
	NEOP	83.81	87.29	95.30	95.13
	LAP	85.41	90.04	92.50	94.40
	LSDSP	88.38	93.22	93.46	94.44
Phage treatment followed by washing treatments	PEO	73.45	75.56	80.59	82.25
	PNEO	70.35	75.22	82.78	85.74
	PLA	70.55	78.81	79.86	85.71
	PLSDS	75.05	77.97	81.37	90.04

EO: Acidic pH EO water with 50 mg/L available chlorine and 2.6 pH, NEO: Neutral pH water with 50 mg/L available chlorine and 6.5pH, P: bacteriophage, EOP: EO treatment followed by P, NEOP: NEO treatment followed by P, LA: 5% lactic acid, LSDS: 1:22 diluted FIT®, LAP: LA treatment followed by P, LSDSP: LSDS treatment followed by P, PEO: P treatment followed by EO, PNEO: P treatment followed by NEO, PLA: P treatment followed by LA, and PLSDS: P treatment followed by LSDS. * Values are average of three repetitions