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Fact Sheet 19-02

Application of bacteriophages on beef trim decreases loads of Shiga Toxin-Producing *E. coli* (STEC) in ground beef

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Summary

A cocktail of seven environmentally-isolated bacteriophages was sprayed on beef trim contaminated with seven strains of Shiga Toxin-Producing *E. coli* (STEC) including the O157:H7 and the "Big Six" (O145, O121, O111, O103, O45, O26). In vitro killing efficiency of an individual phage isolated for each specific strain was: 99.9, 99.6, 96.6, 99.4, 98.6, 96.2, and 99.9 percent for O157:H7, O145, O121, O111, O103, O45, and O26, respectively. When applied on beef trim prior to grinding, bacteriophages reduced up to 0.77 log CFU/g of STEC loads in final ground products. Results indicate that applications of bacteriophages that target O157:H7 and the "Big Six" on beef trim could efficiently improve robust food safety systems to reduce the presence of *E. coli* in ground beef products.

Introduction

Meat products contaminated with STEC, including O157:H7 and the "Big Six" (O145, O121, O111, O103, O45, O26) are considered adulterated by the USDA. These STEC caused 29 percent of the total foodborne illnesses in the U.S. that occurred between 1998 and 20081. *E. coli* O157:H7 alone caused 186 outbreaks from 1998 to 2008, 103 of which were from beef sources (Painter et al., 2013). From 1998 to 2008, STEC O157:H7 caused over 63,000 illnesses, over 2,000 hospitalizations and 20 deaths, whereas non-O157:H7 STEC caused over 112,000 illnesses, over 200 hospitalizations and one death (CDC, 2018). Recently, outbreaks associated with STEC O157:H7 have decreased, however, outbreaks associated with the "Big Six" STEC have increased, raising the question if current interventions used by the meat industry that target non-O157:H7 STEC are efficient. Bacteriophages have been receiving more attention in recent years for their use in food safety due to their precise bacterial strain specificity compared to other food-safety interventions. The Food Safety and Inspection Service (FSIS) directive 7120.1 has approved bacteriophage applications on meats targeting only STEC O157:H7. In this fact sheet, we studied the effects of

bacteriophages on individual STEC in vitro and on contaminated beef.

Procedure

Seven phages (MS1-157:H7, MS1-145, MS1-121, MS1-111, MS1-103, MS1-45 and MS1-26) targeting individual “Big Six” and O157:H7 STECs were isolated from raw sewage using American Type Culture Collection (ATCC) strains recovered from outbreaks. Phages were purified and amplified to 10^{11} PFU/ml. Individual killing efficiency was determined by plating each strain with and without phage application on 1.6 percent LB agar plates. Plates were incubated at 37 C for 24 hours to observe colony formation. For phage efficiency on ground beef, 40 100 g samples of 80 percent lean beef trim were sourced from a USDA inspected facility and randomly assigned to 8 individual treatments: I-6-25 (Inoculated, 6 hours dwell time, 25 C), I-30- (Inoculated, 30 minutes dwell time, 25 C), I-6-7 (Inoculated, 6 hours dwell time, 7 C), I-30-7 (Inoculated, 30 minutes dwell time, 7 C), P-6-25 (Phage treated, 6 hours dwell time, 25 C), P-30-25 (Phage treated, 30 minutes dwell time, 25 C), P-6-7 (Phage treated, 6 hours dwell time, 7 C), and P-30-7 (Phage treated, 30 minutes dwell time, 7 C). The fixed effects included temperature (25 C and 7 C), time (30 minutes and 6 hours), and treatment (Bacteriophage and control).

A STEC inoculum was created by combining each strain in equal amounts when individual strains were at 0.5 to 0.6 absorbance (600 OD), then diluting this solution with buffered peptone water to create a 25 percent STEC cocktail solution that would result in a contamination level of approximately 4 log CFU/g in the ground product. Meat samples were inoculated with the cocktail by uniformly pipetting 2 ml onto the surfaces of the 100 g meat samples. Bacteria was allowed to attach on the meat surface for 30 minutes at 7 C. After attachment, samples were treated with 2 ml BPW (control inoculated) or with 2 ml of bacteriophage solution at 10^{11} PFU/ml by pipetting on the meat surfaces. Samples dwelled for 30 minutes or 6 hours at 7 C or 25°C. Subsequently, the samples were ground with a tabletop electric grinder and an aliquot of 25 g was masticated in 225 ml BPW for 2 minutes at 230 rpm. Ten (10) ml of the homogenate was centrifuged at 10,000 x g for 6 minutes and the supernatant was discarded to avoid plating phages. The pellet was resuspended in 10 ml BPW and serially diluted before plating on LB agar. Controls were diluted to 10^{-4}, and phage treatments were diluted to 10^{-3}, before 0.1 ml of each dilution was plated in duplicate. Plates were inverted and incubated at 37 C for 24 hours and resulting colonies were counted. Data were analyzed using SAS as a completely randomized design and contrasts between phage treatment, lysing time, and temperature were evaluated.

Results

Table 1 illustrates the killing efficiency of each bacteriophage against STEC in vitro. Overall, phages targeting their specific STEC sowed at least a 96.2 percent of lysing efficiency rate, whereas some of them showed higher or lower lysing interspecificity (efficiency in killing other strain, e.g. MS1-O157:H7 interspecific lysing rate was higher than MS1-O45 rate).

In ground beef we observed an overall significant decrease of approximately 1.5 log when comparing I-6-25 (4.28 log CFU/g) versus P-6-7 (2.81 log CFU/g) treatments. In total, 6 hours at 25 C, 30

minutes at 25 C, 6 hours at 7 C, 30 and minutes at 7 C reduced by 0.69, 0.77, 0.6 and 0.73 log CFU/g respectively. The contrast analysis revealed significant effects of phage application (*P*=0.0005) and temperature *(P*=0.0339), but no significant effect from time (*P*=0.2134). Within same temperature conditions, phage applications led to a significant 0.70 log reduction when lysing time was 30 minutes at 7 C and 0.77 log reduction when lysing time was 30 minutes at 25 C.

Conclusion

Bacteriophage applications decrease loads of all STEC deemed adulterant by the USDA. Phages targeting non-O157:H7 strains are effective in lowering the “Big Six” strains including the O145, O121, O111, O103, O45 and O26 strains. Phage replication and lysing ability depends on cytoplasmic machinery of the bacteria which is related to the surrounding temperature. The metabolic rates of STECs and phages are proportionately related to temperature, where bacteria replication and phage lysing activity seem to be proportionally higher as temperature increases.

Implications

The FDA must further consider giving bacteriophage applications targeting non-O157:H7 strains a GRAS (Generally Recognized as Safe) status. This will allow meat processing facilities to include an additional food safety intervention into existent robust systems.

References

CDC, Centers for Disease Control and Prevention. Timeline for Reporting Cases of E. coli O157 Infection. (2018). https://www.cdc.gov/ecoli/reporting-timeline.html

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J....Griffin, P. M. (2013). Attribution of Foodborne Illnesses, Hospitalizations, and Deaths to Food Commodities by using Outbreak Data, United States, 1998–2008. Emerging Infectious Diseases, 19(3), 407-415. https://dx.doi.org/10.3201/eid1903.111866.

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Table 1. Lysing efficiency[1] of individual bacteriophages MS1-157:H7, MS1-145, MS1-121, MS1-111, MS1-103, MS1-45 and MS1-26 against STEC O157:H7 (ATCC® 35150™), O26 (ATCC® BAA-2196™), O45 (ATCC® BAA-2193™), O103 (ATCC® BAA-2215™), O111 (ATCC® BAA-2440™), O121 (ATCC® BAA-2219™), and O145 (ATCC® BAA-2192™) in vitro.

	Bacteriophages						
STEC	**MS1-O157:H7**	**MS1-O145**	**MS1-O121**	**MS1-O111**	**MS1-O103**	**MS1-O45**	**MS1-O26**
O157:H7	99.9%	96.5%	99.1%	50.9%	45.8%	54.9%	77.2%
O145	98.8%	99.6%	97.1%	89.0%	84.0%	62.8%	96.7%
O121	87.0%	88.0%	96.6%	85.6%	79.1%	73.4%	58.5%
O111	98.8%	99.9%	99.3%	99.4%	34.1%	82.2%	67.3%
O103	99.7%	97.4%	79.7%	97.0%	96.2%	55.6%	93.7%
O45	99.4%	96.2%	88.5%	93.3%	95.8%	98.6%	68.4%
O26	99.8%	96.1%	95.6%	97.6%	80.7%	58.7%	99.9%

Table 2. Effects of bacteriophage applications on STEC loads in beef held for 30 m and 6 h at 7°C and 25°C (Log CFU/g).

Treatment	Control	Bacteriophage	Log CFU/g reduction
7 C at 30 minutes	4.13	3.40	0.73
7 C at 6 hours	3.41	2.81	0.60
25 C at 30 minutes	4.11	3.34	0.77
25 C at 6 hours	4.28	3.59	0.69