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Efficiency of Environmentally Isolated Bacteriophages against the ‘Big Six’ and O157:H7 Stecs in Ground Beef

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Objectives

Phages targeting *E. coli* O157:H7 are approved by the FSIS (directive 7120.1) for application on beef trim. This study was conducted to evaluate the efficiency of 7 environmentally isolated phages against “Big Six” and O157:H7 strains in vitro and in ground beef.

Materials and Methods

Seven phages (MS1–157:H7, MS1–145, MS1–121, MS1–111, MS1–103, MS1–45, and MS1–26) targeting individual non-O157:H7 and O157:H7 STECs were isolated from raw sewage using ATCC strains recovered from outbreaks. Phages were purified and amplified to 10^8 to 10^9 PFU/ml. Individual killing efficiency was determined by plating each strain with and without its respective phage on LB agar. For ground beef, 40 ($n = 40$) 100-g samples of 80% lean trim were randomly assigned to 8 treatments including I-6–25 (Inoculated, 6 h of lysing time at 25°C), I-30–25 (Inoculated, 30 min of lysing time at 25°C), I-6–7 (Inoculated, 6 h of lysing time at 7°C), I-30–7 (Inoculated, 30 min of lysing time at 7°C), P-6–25 (Phage treated, 6 h of lysing time at 25°C), P-30–25 (Phage treated, 30 min of lysing time at 25°C), P-6–7 (Phage treated, 6 h of lysing time at 7°C), and P-30–7 (Phage treated, 30 min of lysing time at 7°C). After bacterial attachment of 30 min at 7°C, samples were treated with the phage cocktail solution at 10^9 PFU/ml. Phages were allowed to lyse bacteria for 30 min and 6 h at 7°C and 25°C. Subsequently, trim was ground and an aliquot of 25 g was stomached in BPW. The pellet was resuspended in BPW and serially diluted before plating on LB agar. Plates were incubated

at 37°C for 24 h and colonies were enumerated. Data were analyzed using SAS as a completely randomized design and contrasts between phage treatment, lysing time, and temperature were evaluated.

Results

Killing efficiency of each phage isolated for specific strain was: 99.9, 99.6, 96.6, 99.4, 98.6, 96.2, and 99.9% for O157:H7, O145, O121, O111, O103, O45, and O26, respectively. Inoculated meat samples yielded approximately 4 log CFU/g in the ground product. Overall, a significant decrease of approximately 1.5 log was observed when comparing I-6–25 (4.28 log CFU/g) versus P-6–7 (2.80 log CFU/g) treatments. The contrast analysis revealed significant effects of phage application ($P = 0.003$) and temperature ($P = 0.03$). Within same temperature conditions, phage applications led to a significant 0.70 log reduction when lysing time was 30 min at 7°C and 0.77 log reduction when lysing time was 30 min at 25°C.

Conclusion

Application of a cocktail of phages targeting all adulterant strains on trim prior to grinding decreased STEC populations in ground beef. Metabolic rates of STECs and phages are affected by temperature where bacterial replication and phage lysing activity seem to increase proportionally with temperature. Overall, phages that target adulterant STECs including O157:H7 and “Big Six” strains, could provide an additional barrier against these pathogens in robust food safety systems.